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Few studies have examined the partitioning of organic matter in upwelling systems, despite the fact that these systems play a key role in carbon and nitrogen budgets in the ocean. We examined the production and partitioning of phytoplankton-derived organic matter in deck incubations off Oregon during the upwelling season. During exponential growth of the phytoplankton, $\geq$ 78% of total accumulated organic matter was in particulate form. A small accumulation of dissolved organic matter (DOM) was only observed in one incubation and the percentage of daily primary production released extracellularly averaged 12.7 $\pm$ 2.75 %, suggesting that DOM is a small fraction of primary production during exponential growth of coastal phytoplankton blooms. Following nitrate depletion, carbon-rich (C:N $\geq$ 16) DOM accumulated and the percentage of daily primary production released as dissolved organic carbon (DOC) averaged 57.7 $\pm$ 10.7 %. Abundances and growth rates of bacteria with a high DNA content increased rapidly concomitant with the large DOM release, while little response was observed from bacteria with a low DNA content. Despite the enhanced bacterial growth, a net decrease in DOC was only observed in one incubation and immediately

after reaching maximum abundance, high-DNA bacterial abundances declined while heterotrophic nanoflagellate abundances increased. These results indicate that both bottom- up and top- down controls may act to prevent bacterial degradation of phytoplankton DOM, thus allowing the short-term accumulation (several weeks) of C rich DOM in the Oregon upwelling system. In addition to the accumulation of C rich DOM, approximately 70 to 157% more C was fixed than would be predicted by Redfield stoichiometry based on measured concentrations of nitrate and accumulated TOC. Accumulation of C rich DOM and excess carbon fixation suggests that nitrate assimilation (i.e., new production) might not equate to net production of POM in coastal upwelling systems.

Production and Partitioning of Organic Matter During Simulated Phytoplankton Blooms

by
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Michael S. Wetz, Author

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CONTRIBUTION OF AUTHORS

Dr. Patricia A. Wheeler assisted with the experimental design in Chapters 2 and 3, and provided critiques that greatly improved the manuscripts found in those chapters as well.

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INTRODUCTION

Eastern boundary current upwelling systems are among the most productive marine ecosystems on the planet. Although coastal upwelling systems account for < 1 % of global ocean surface area, they account for a disproportionate amount of annual marine primary production. The intense primary production in these regions is driven by unique atmospheric-oceanic interactions. Off Oregon for example, a strong high-pressure system develops in the north Pacific Ocean in the spring and summer. This causes a general pattern of northerly or "upwelling-favorable" winds along the coast. Once upwelling-favorable winds develop, the ocean responds within a day (Smith 1995) and offshore Ekman transport of surface waters (usually upper 20 m) begins to occur. The result is pumping of cool, nutrient-rich deep water to the surface and subsequent transport of that water mass away from the upwelling front (Figure 1.1). The nutrient- rich water,

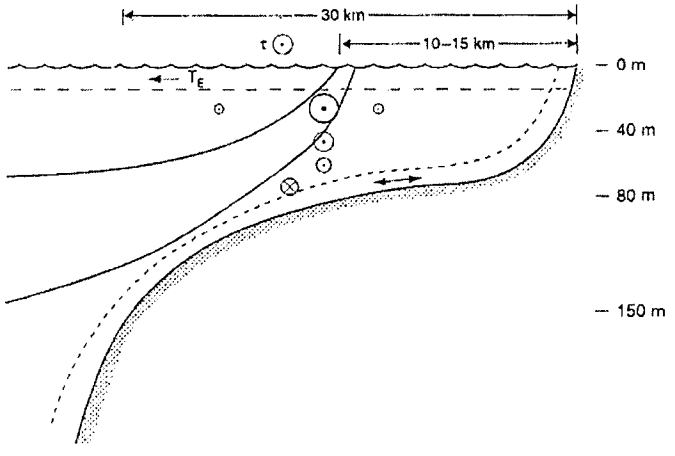

Figure 1.1. Schematic of upwelling circulation off northern Oregon. Arrowheads (tails) indicate southward (northward) flow. Diagram courtesy of Dr. Jack Barth.

in conjunction with stabilization of the upper water column, spurs rapid phytoplankton growth. In a typical year, approximately five large upwelling "events" and many smaller events occur. Upwelling events off Oregon typically last up to two weeks and are terminated when winds reverse to downwelling favorable, which breaks down stratification of surface waters (Smith 1995).

Although it is widely recognized that coastal upwelling systems are sites of intense primary production, surprisingly little is known about their role in global carbon and nitrogen cycles. For instance, estimates of global new production (defined mainly as nitrate based production in coastal systems) range from ~ 3 to 22 Gt C y^{-1} (reviewed by Chavez and Toggweiler 1995). For coastal upwelling systems, estimates based solely on physical inputs of nitrate to the euphotic zone and assuming Redfield stoichiometry range from 0.8 to 1.2 Gt C y^{-1} (Walsh 1991; Chavez and Toggweiler 1995). This range seems small, but in actuality represents a large percentage of the lower estimates of global new production, and even for the more recent estimate of 7.2 Gt C y^{-1} (Chavez and Toggweiler 1995), it represents a 6 % uncertainty. The variability in new production estimates is due to the difficulty of estimating annual water flows and surface areas for upwelling systems. In addition, the use of Redfield stoichiometry may further confound estimates. Although nitrate-based estimates factor out methodological problems associated with measuring primary production, there can be problems inherent with use of nitrate assimilation. Redfield stoichiometry relating carbon fixation to nitrate assimilation is often not followed *in situ*, and thus it may only provide a first order estimate of new production. For example, nitrogen stress in phytoplankton often results

in production of carbon- rich storage products and an increase in the cellular C:N ratio (reviewed by Turpin 1991). This could explain deviations in the C:N ratio of particulate organic matter (POM) from Redfield that have been observed *in situ*, particularly during bloom decay (reviewed by Geider and La Roche 2002). Additionally, production of usually C- rich dissolved organic matter (DOM) can complicate new production estimates. In order to improve upon previous new production estimates for coastal upwelling systems, we need to know more about production of both POM and DOM in relation to nitrate supply.

Accumulation of DOM introduces more complexity to food-web dynamics and an alternate pathway for export of organic matter from coastal systems. DOM is a chemically complex pool consisting of numerous types of molecules, only a small portion of which have been characterized (Benner 2002). The chemical composition of DOM will have a major impact on its biological availability and is strongly related to the source of the material. Labile compounds such as free amino acids and sugars can be assimilated or respired rapidly (hours to days) by microbial communities. Bacterioplankton and small phytoplankton associated with microbial food webs are generally ignored in export estimates due to the fact that they contribute a minor fraction of the sinking POM (e.g. Eppley and Peterson 1979). In contrast, semi-labile compounds such as carbohydrates and refractory, highly degraded DOM are resistant to rapid microbial degradation. Thus, they can be exported from the euphotic zone through horizontal advection, vertical mixing, or sinking of aggregates (Peltzer and Hayward

1996; Passow 2000), possibly resulting in long-term removal of C and N from the euphotic zone.

There are many sources of DOM in the marine environment, including phytoplankton exudation (Hellebust 1965), grazing (Strom et al. 1997), viral lysis (Bratbak et al. 1990), bacterial degradation of detritus or aggregates (Biddanda 1988; Smith et al. 1992), and terrestrial input (Hill and Wheeler 2002). In most marine systems, including coastal upwelling regions, the relative contribution of the various sources is largely unknown. However, recent studies have shown that autochthonously produced DOM may be an important component of C and N budgets in upwelling systems (Álvarez-Salgado et al. 2001; Hill & Wheeler 2002).

In this study, water samples were collected from the northern and central Oregon shelf (Figure 1.2) and incubated to simulate upwelling phytoplankton blooms. The main goal was to observe how organic carbon and nitrogen were partitioned during the blooms. Also, we wanted to begin to address the fate of nitrate-based production, as nitrate assimilation is frequently used as a proxy for new production in upwelling systems (Eppley and Peterson 1979). Chapter 2 focuses on the partitioning of the organic matter and also on the total amount of organic matter produced in relation to nitrate assimilation. Chapter 3 deals with the bacterial response to the phytoplankton blooms, and in particular focuses on top-down and bottom-up controls of bacterioplankton growth. Finally, Chapter 4 synthesizes the results of this study and discusses several aspects of this study that necessitate future work.

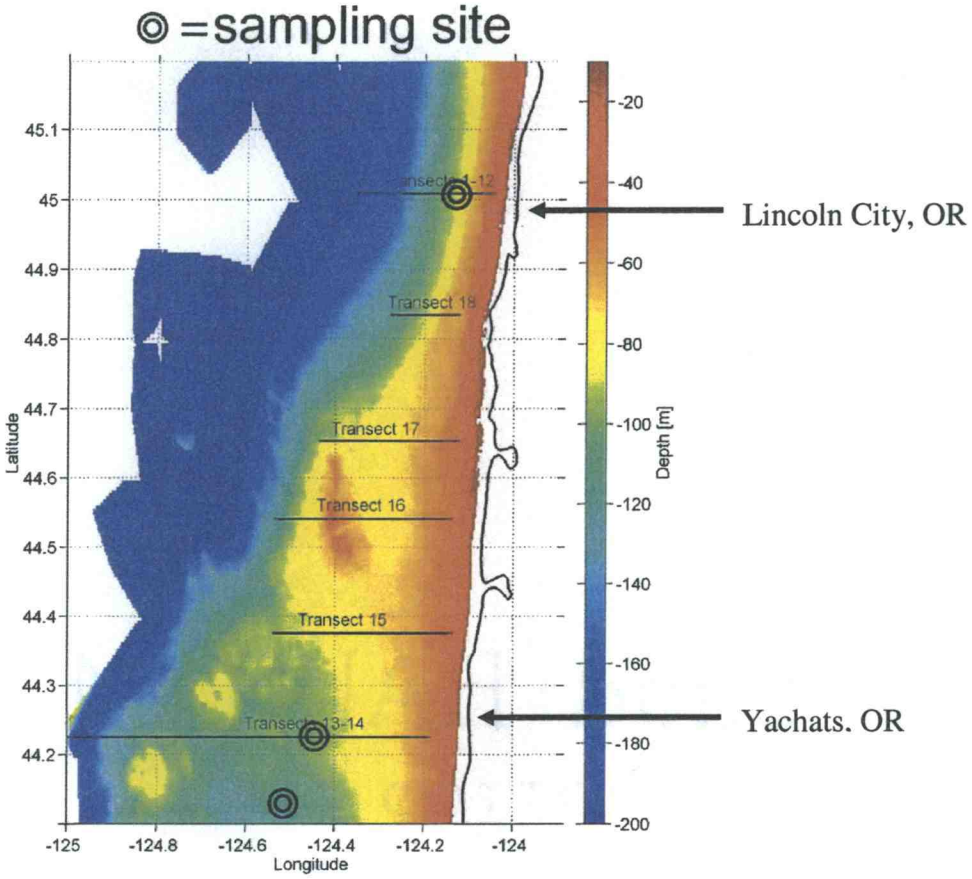

Figure 1.2. Map of study site off Oregon. Circles indicate where water samples were collected for deck incubations.

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ABSTRACT

Few studies have examined the partitioning of organic matter in upwelling systems, despite the fact that these systems play a key role in carbon and nitrogen budgets in the ocean. We examined the production and partitioning of phytoplankton-derived organic matter in deck incubations off Oregon during the upwelling season. During exponential growth of the phytoplankton, ≥ 78% of total accumulated organic matter was in particulate (POM) form. This suggests that DOM is a small fraction of primary production during exponential growth of coastal phytoplankton blooms. Following nitrate depletion, carbon-rich (C:N ≥ 16) dissolved organic matter (DOM) accumulated in incubations dominated by the diatom *Chaetoceros* sp., accounting for 38 ± 8.5% of accumulated total organic carbon (TOC) and 24 ± 8% of accumulated total organic nitrogen (TON). However, in a bloom dominated by the diatom *Leptocylindrus minimus*, a relatively smaller amount of DOM accumulated, accounting for only 15% of accumulated TOC and 7% of accumulated TON. Based on measured concentrations of nitrate and accumulated TOC, approximately 70 to 157% more carbon was fixed than would be predicted by Redfield stoichiometry (referred to as "excess carbon fixation"), with 20 to 69% of the excess carbon fixation occurring after nitrate depletion. Accumulation of carbon-rich DOM and excess carbon fixation suggests that nitrate assimilation (i.e., new production) might not equate to net production of POM in coastal upwelling systems.

INTRODUCTION

Coastal upwelling systems are among the most productive marine ecosystems in the world. Although accounting for only 1% of total ocean surface area, coastal upwelling regions account for ≥ 10% of global new production (Chavez and Toggweiler 1995). New production is defined as primary production that is based on newly available nitrogen (Dugdale and Goering 1967) and is commonly determined from regional observations of nitrate-based primary production, as nitrate is globally the main source of new nitrogen to the euphotic zone. In coastal regions, the fate of new production is thought to be export through sinking of particulate organic matter (POM) or through accumulation of higher trophic level biomass (e.g. fish production) (Eppley and Peterson 1979; Walsh 1991). However, earlier paradigms are now being revisited as numerous studies have shown accumulation of dissolved organic matter (DOM) following phytoplankton blooms and over the course of the growing season in a variety of marine systems (Ittekkot et al. 1981; Bronk et al. 1994; Williams 1995). Additionally, elevated carbon fixation relative to nitrogen assimilation has been observed in the surface ocean (Sambrotto et al. 1993). The fate of this excess fixed carbon is largely unknown. Mechanisms, such as aggregate formation resulting from transparent exopolymer (TEP) production by phytoplankton, are now being elucidated and these might facilitate rapid export of the excess fixed carbon from the euphotic zone (Engel et al. 2002).

Until recently, the marine DOM pool has received relatively little attention because of its complexity. There are many sources of DOM in the marine environment, including phytoplankton exudation (Hellebust 1965), grazing (Strom et al. 1997), viral lysis

(Bratbak et al. 1990), bacterial degradation of detritus or aggregates (Biddanda 1988; Smith et al. 1992), and terrestrial input (Hill and Wheeler 2002). In most marine systems, including coastal upwelling regions, the relative contribution of the various sources is largely unknown. In addition to having multiple sources, DOM is a chemically complex pool with only a small portion characterized (Benner 2002). The chemical composition of DOM will have a major impact on its biological availability and is strongly related to the source of the material. Labile DOM can be assimilated or respired rapidly (hours to days) by microbial communities, which are generally ignored in export estimates due to the fact that they contribute a minor fraction of the sinking POM (e.g. Eppley and Peterson 1979). In contrast, semi-labile DOM, or DOM that is resistant to rapid microbial degradation, can be exported from the euphotic zone through horizontal advection, vertical mixing, or sinking of aggregates (Peltzer and Hayward 1996; Passow 2000), possibly resulting in long-term removal of C and N from the euphotic zone.

Along with the lack of information concerning sources and chemical composition of DOM, there is relatively little information concerning spatial and temporal distribution of DOM and POM, particularly in upwelling systems. In a broad spatial survey conducted in Oregon coastal waters during the upwelling season, highest concentrations of chlorophyll *a* (ca. 10 $\mu g\ L^{-1}$) and POM (50 to 70 $\mu mol\ L^{-1}$ POC; 7 to 8 $\mu mol\ L^{-1}$ PON) were found in coastal waters influenced by upwelling (Hill 1999; Hill and Wheeler 2002). High DOC concentrations ($\geq$ 130 $\mu mol\ L^{-1}$) were found in both Columbia River plume water and in shelf waters offshore of the upwelling front, while highest DON concentrations (7 to 10 $\mu mol\ L^{-1}$ DON) were found in shelf waters offshore of the

upwelling front. During a subsequent time-series study at a coastal Oregon site influenced by upwelling, Hill and Wheeler (2002) found that POM and DOM concentrations were highest during the upwelling season, suggesting that phytoplankton were a major source of the organic matter. Roughly 63% of the organic carbon produced in excess of deep water concentrations was as DOC, indicating a potentially pronounced role for DOM in terms of the fate of new production in the Oregon upwelling system.

In this study, production and partitioning of organic carbon and nitrogen were examined in deck incubations. The goals of this study were two-fold. First, we wanted to document how organic matter is partitioned in upwelling-induced phytoplankton blooms. Development of blooms with different phytoplankton community compositions in the incubations facilitated exploration of the effects of species composition on organic matter production and partitioning. We also wanted to begin to address the fate of nitrate-based production, as nitrate assimilation is frequently used as a proxy for net primary production in upwelling systems (Eppley and Peterson 1979). Whether newly produced organic matter accumulates primarily as DOM or POM will have profound implications for food-web structure and for understanding carbon and nitrogen cycles in the ocean.

METHODS

Experiments were run from 6 to 25 August 2001. Water samples for two incubations (#1 and #2; A and B on all figures) were collected from mid-shelf sites on a transect off northern Oregon (45.00 N, 124.02 W) where the shelf and region of active upwelling are

relatively narrow. Water samples for the other two incubations (#3 and #4; C and D on all figures) were collected from mid-shelf sites on transects off central Oregon (44.13 N, 124.28 W and 44.06 N, 124.33 W respectively) in an area characterized by a broad shelf. Maps of the study region can be viewed at http://damp.oce.orst.edu/coast/summary.shtml.

For each incubation, triplicate 20-L high density polyethylene (HDPE) Cubitainers™ were filled with 1 L of surface water inoculum and 19 L of water from below the mixed layer (ca. 28 to 70 m). Seawater was pumped directly into the Cubitainers™ from depth using a towed sled with a pump attached. Prior to being filled, Cubitainers™ were vigorously rinsed with 10% HCl followed by multiple rinses with deionized water (DIW). All incubations began in the early evening (0100 to 0400 GMT). Cubitainers™ were held in a deck incubator at in situ surface water temperatures ranging from 11 to 15.5^0 C, with an average of 13.5^0 C. There were approximately 14 hours of light and 10 hours of darkness daily during the cruise and daily integrated PAR values at the surface ranged from 15.0 to 52.0 mol quanta m^{-2} d^{-1} (mean = 31.6 ± 12.0 mol quanta m^{-2} d^{-1}). The incubator was covered with one layer of neutral density mesh screen that reduced light intensities to ca. 50% of surface intensities. Moderate mixing of samples occurred due to the motion of the boat, and the samples were vigorously mixed manually once or twice daily. Cubitainers™ were sampled daily in the early morning (1400 to 1700 GMT). Incubations lasted 7 to 8 days.

Biological analyses - Three different size fractions of chlorophyll were collected; whole water, < 20 μm, and < 3 μm. Whole water samples were vacuum filtered (< 200 mm Hg) onto GF/F filters. Samples for < 20 μm chlorophyll were gravity filtered

through a 20 μm mesh screen and then vacuum filtered onto GF/F filters. Samples for < 3 μm chlorophyll were first gravity filtered through a 20 μm mesh screen, then vacuum filtered through a 3 μm polycarbonate filter (< 200 mm Hg) and finally vacuum filtered onto a GF/F filter. After filtration, all samples were stored in glass Vacutainers™ and immediately frozen at -30^0 C until laboratory analysis. Chlorophyll *a* was extracted from the filters for ≥12 h in the dark at –20^0 C using 95% methanol. Fluorescence was measured with a Turner 10-au fluorometer. For the 3 to 20 μm size fraction, standard deviations were calculated by propagation of error using standard deviations from < 20 μm, and < 3 μm chlorophyll size fractions (Bevington 1969). For the > 20 μm size fraction, standard deviations were calculated by propagation of error using standard deviations from < 20 μm and whole water chlorophyll size fractions.

Chemical analyses - Nutrient samples were collected in acid-washed 30 ml HDPE bottles and immediately frozen at –30^0 C. Samples were processed within 4 months of collection. Samples were analyzed on a Technicon AA-II according to standard wet chemical methods of Gordon et al. (1995). Standard curves with four different concentrations were run daily at the beginning and end of each run. Fresh standards were made prior to each run by diluting a primary standard with low nutrient surface seawater. DIW was used as a blank, and triplicate DIW blanks were run at the beginning and end of each run in order to correct for any baseline shifts. Nitrate was determined by subtracting nitrite values from nitrate plus nitrite (N+N) values. The standard deviation for nitrate was calculated by propagation of error using standard deviations for N+N and nitrite. Analytical error, represented as the deviation of each point of the standard curve from a

linear regression line, was 0.02 ± 0.03 μmol L^{-1} for phosphate, 0.05 ± 0.03 μmol L^{-1} for ammonium, 1.28 ± 0.78 μmol L^{-1} for silicate, 0.26 ± 0.17 μmol L^{-1} for N+N, and 0.02 ± 0.01 μmol L^{-1} for nitrite. Nutrient usage ratios are the slopes of nitrate vs. phosphate or nitrate vs. silicate prior to the first day of nitrate depletion, and were determined from simple linear regression models using the least squares method for estimating the slope. Significance levels of the slopes were determined using two-sided t-tests.

Total nitrogen (TN) samples were collected in acid washed 60 ml HDPE bottles and immediately frozen at -30^0 C until laboratory analysis. Samples were processed within five months of collection, and were analyzed over three days. Organic nitrogen was converted to nitrate using a persulfate wet oxidation method (Libby and Wheeler 1997), which was then analyzed using a Technicon AA-II. Instrument calibration was performed daily using a standard curve prepared from triplicate digested leucine standards at three concentrations. Fresh standards were made prior to each run by diluting a primary standard with artificial seawater. Digested artificial seawater was used as a blank, and the standard curve was corrected for nitrogen content of the blank by determining the concentration of nitrogen in the persulfate solution and then calculating the amount of nitrogen in the artificial seawater. Artificial seawater nitrogen content was estimated as the difference between blank signal and persulfate signal. Average coefficient of variation for all TN replicates was 2.61 ± 0.33%, and the highest observed coefficient of variation was 8.02%.

Total organic carbon (TOC) samples were collected in borosilicate vials with Teflon cap liners. Each vial contained approximately 5 ml of seawater that was preserved with

50 μl of 90% phosphoric acid. Samples were stored at room temperature until being processed 3 months later. Samples were analyzed over a three day period using the High Temperature Catalytic Combustion method on a Shimadzu TOC-5000A analyzer. Standard curves were run twice daily using a DIW blank and four concentrations of an acid potassium phthalate solution. Five subsamples were taken from each water sample and injected in sequence. Variance between subsamples was $\leq 5.26\%$ (mean = $2.62 \pm 1.19\%$). Deep-water standards of known TOC concentration were injected after every three to four samples (fifteen to twenty subsamples) to check for baseline shifts. For the three days of sample analysis, average daily TOC concentrations in the deep-water standard were 38.9, 40.1, and 41.7 μmol L^{-1}. Baseline drift was calculated from changes in the deep-water concentrations during a run, and a drift correction was applied to the raw data. The data were then normalized to a long-term average TOC concentration of the deep-water standard (~ 40.1 μmol L^{-1}). As an additional check to make sure the system was working properly, Sargasso Sea deep-water standard (obtained from Dr. W. Chen, Certified Reference Materials Program, University of Miami) was injected once or twice during each run. Average TOC concentration in the Sargasso Sea deep-water for the three runs was 45.2 ± 1.1 μmol L^{-1}, indicating little systematic variation from day to day. When the coefficient of variation of the triplicates was > 15%, a sample value was removed if it was greater than one standard deviation away from the mean of the triplicates. Only one value was excluded out of ninety measurements total. Average coefficient of variation for all TOC replicates was $4.84 \pm 0.64\%$, and no replicates had a coefficient of variation higher than 12.7%.

Particulate organic carbon (POC) and nitrogen (PON) were determined from material collected on precombusted GF/F filters. Water samples were pre-filtered through a 202 μm mesh screen to remove large zooplankton. Then, 500 or 1000 ml of the filtered water was vacuum filtered (< 200 mm Hg) onto precombusted GF/F filters. After filtration, samples were stored in glass Vacutainers^{TM} and immediately frozen at -30^0 C until laboratory analysis. Samples were processed within 2 months of collection. Filters were fumed with hydrochloric acid to remove inorganic carbon and dried, followed by analysis using a Control Equipment Corp. 440HA CHN elemental analyzer calibrated with acetanilide. During analysis, filter blanks were run after every nine to ten samples. Filter blank averages were 18.4 ± 3.26 μg C and 0.33 ± 1.08 μg N, and these values were subtracted from each measured value as a filter blank correction. Carbon filter blanks averaged 24% of initial (day 0) POC samples and 2% of maximum POC samples for the four incubations. Nitrogen filter blanks were $\leq$ 6% of all PON samples. When the coefficient of variation of the triplicates was > 15%, a sample value was removed if it was greater than one standard deviation away from the mean of the triplicates. In all, six POC and three PON values were removed out of a total of eighty-nine measurements. Average coefficient of variation for all POC and PON replicates was 6.48 ± 0.68% and 5.49 ± 0.64% respectively, and the highest observed coefficient of variation for POC and PON replicates was 13.3% and 12.5% respectively.

Dissolved organic nitrogen (DON) was determined by subtracting PON and DIN (NH_4, NO_3^-, and NO_2^-) values from TN values, as shown in Eq. (1).

$$DON = TN - PON - DIN \qquad (1)$$

The standard deviation for DON was calculated by propagation of error using standard deviations for TN, PON, and DIN. Average coefficient of variation for all DON replicates was 17.1 ± 2.14%.

Dissolved organic carbon (DOC) was determined by subtracting POC values from TOC values, as shown in Eq. (2).

$$DOC = TOC - POC \qquad (2)$$

The standard deviation for DOC was calculated by propagation of error using standard deviations for TOC and POC. Average coefficient of variation for all DOC replicates was 13.8 ± 1.96%. Most of the variation came from samples where TOC concentrations were > 100 μmol L^{-1}.

RESULTS

Phytoplankton biomass, size-structure, and nutrient usage - Phytoplankton blooms developed in all four incubations and chlorophyll *a* peaked in 4 to 6 days depending on initial chlorophyll concentrations (Fig. 2.1). Maximum chlorophyll *a* concentrations ranged from 23 to 41 μg Chl *a* L^{-1}. The first three blooms were composed of large (>20 μm) cells (Figs. 2.1A-C), namely the diatom *Chaetoceros sp.*. The last bloom was composed primarily of the small (~ 12 x 3 μm) diatom *Leptocylindrus minimus* (ca. 60% of peak chlorophyll *a*) (Fig. 2.1D), although some larger diatoms were present (ca. 30% of peak chlorophyll *a*). Initial nitrate values ranged from 25 to 34 μmol L^{-1}. The ratio of nitrate to phosphate used ranged from 13.1 to 15.6 (mean = 14.6 ± 0.55) (Table 2.1). Nitrate was drawn down below detection limits (~ 0.26 μmol L^{-1}) in each incubation, but

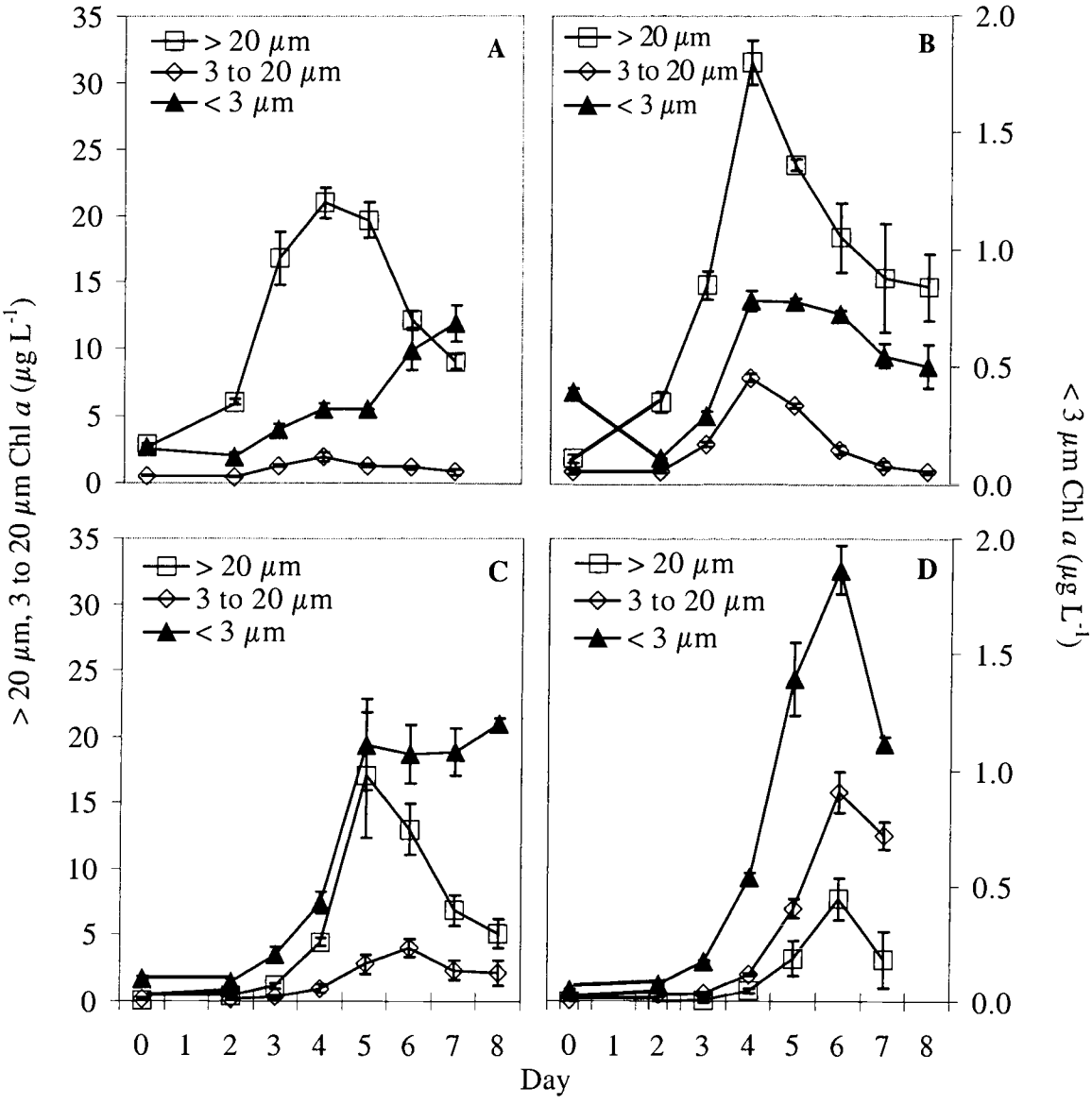

Figure 2.1. Size-fractionated chlorophyll *a* concentrations in deck incubations. Panels A and B correspond to incubation #1 and #2 from sites off northern Oregon, while panels C and D correspond to incubation #3 and #4 from sites off central Oregon. Error bars represent standard deviations.

Table 2.1. Nutrient concentrations (μmol L^{-1}) and nutrient usage ratios in deck incubations. Values designated "nd" indicate that the sample was below limits of detection based on analytical error estimates (see Methods). Ratios of nutrients used are the slopes of linear regressions of nitrate vs. phosphorous and nitrate vs. silicate prior to the first day of nitrate depletion, including data from all three replicate carboys. In some cases, only duplicate nitrate values were available.

Inc. #	Day	NO_3^- ± SD	NO_2^- ± SD	NH_4^+ ± SD	$Si(OH)_4$ ± SD	PO_4^{3-} ± SD	N:P Used ± SE 95% Conf. Int.	N:Si Used ± SE 95% Conf. Int.
1	0	30.79 ± 2.05	0.29 ± 0.02	1.08 ± 0.35	43.68 ± 3.77	2.64 ± 0.14	14.62 ± 0.32	1.21 ± 0.09
	2	28.35 ± 0.37	0.35 ± 0.00	nd	41.28 ± 0.11	2.41 ± 0.02	13.86 to 15.38	0.99 to 1.42
	3	15.41 ± 1.44	0.40 ± 0.01	0.15 ± 0.09	31.61 ± 1.41	1.57 ± 0.10	n = 9	n = 9
	4	nd	0.12 ± 0.00	0.34 ± 0.07	3.40 ± 1.79	0.46 ± 0.01	$p < 0.01$	$p < 0.01$
	5	nd	0.13 ± 0.00	0.25 ± 0.00	3.96 ± 6.12	0.70 ± 0.08		
	6	nd	0.07 ± 0.00	0.69 ± 0.71	nd	0.71 ± 0.06		
	7	nd	0.06 ± 0.01	0.28 ± 0.04	nd	0.70 ± 0.01		
2	0	30.99 ± 0.27	0.38 ± 0.00	2.85 ± 0.02	48.65 ± 0.27	3.01 ± 0.01	13.07 ± 0.36	0.84 ± 0.02
	2	28.92 ± 0.28	0.40 ± 0.00	1.16 ± 0.20	43.85 ± 0.70	2.77 ± 0.03	12.25 to 13.90	0.78 to 0.89
	3	21.71 ± 0.40	0.42 ± 0.00	0.09 ± 0.05	35.25 ± 0.15	2.11 ± 0.05	n = 10	n = 10
	4	0.26 ± 0.07	0.14 ± 0.00	0.40 ± 0.03	10.58 ± 1.22	0.65 ± 0.02	$p < 0.01$	$p < 0.01$
	5	nd	0.15 ± 0.01	0.39 ± 0.03	nd	0.76 ± 0.06		
	6	nd	0.07 ± 0.00	0.31 ± 0.03	nd	0.69 ± 0.10		
	7	nd	0.06 ± 0.00	0.29 ± 0.04	nd	0.67 ± 0.02		
	8	nd	0.05 ± 0.01	0.40 ± 0.28	1.35 ± 0.88	0.72 ± 0.15		
3	0	34.27 ± 0.47	0.16 ± 0.03	nd	49.36 ± 0.31	2.80 ± 0.03	15.04 ± 0.39	1.69 ± 0.13
	2	33.35 ± 0.60	0.17 ± 0.02	0.16 ± 0.28	47.58 ± 2.54	2.75 ± 0.02	14.19 to 15.89	1.41 to 1.98
	3	32.42 ± 0.54	0.21 ± 0.01	0.07 ± 0.04	49.54 ± 0.58	2.57 ± 0.04	n =15	n =15
	4	28.46 ± 0.47	0.17 ± 0.02	0.06 ± 0.05	47.18 ± 0.55	2.27 ± 0.06	$p < 0.01$	$p < 0.01$
	5	6.79 ± 3.41	0.21 ± 0.06	0.16 ± 0.02	34.54 ± 4.27	0.95 ± 0.21		
	6	nd	0.06 ± 0.00	0.58 ± 0.30	17.05 ± 4.73	0.69 ± 0.06		
	7	nd	0.05 ± 0.00	0.65 ± 0.28	10.95 ± 2.96	1.06 ± 0.14		
	8	nd	0.05 ± 0.00	0.28 ± 0.19	9.25 ± 2.70	0.89 ± 0.06		
4	0	24.98 ± 0.32	0.15 ± 0.00	0.06 ± 0.04	29.31 ± 0.27	1.99 ± 0.02	15.63 ± 0.23	2.55 ± 0.28
	2	24.56 ± 0.38	0.16 ± 0.00	nd	28.71 ± 0.97	1.93 ± 0.05	15.12 to 16.14	1.93 to 3.17
	3	23.72 ± 0.50	0.18 ± 0.00	nd	28.74 ± 0.70	1.92 ± 0.02	n =12	n =12
	4	21.65 ± 0.13	0.12 ± 0.00	nd	28.09 ± 0.56	1.74 ± 0.04	$p < 0.01$	$p < 0.01$
	5	12.05 ± 1.04	0.15 ± 0.02	nd	24.86 ± 1.48	1.17 ± 0.04		
	6	nd	0.06 ± 0.00	0.20 ± 0.00	13.33 ± 0.16	0.38 ± 0.02		
	7	nd	0.06 ± 0.00	0.21 ± 0.03	8.39 ± 0.43	0.51 ± 0.05		

phosphate was never depleted. The ratio of nitrate to silicate used ranged from 0.84 to 1.69 (mean = 1.24 ± 0.25) in the microplankton blooms. However, the *Leptocylindrus minimus* bloom used approximately 2.55 μmol nitrate per μmol silicate (Table 2.1). Chlorophyll *a* in the > 20 μm size fraction began to decline immediately following nitrate depletion in all four incubations. However, chlorophyll in the < 3 μm size fraction remained elevated or increased in two of the incubations following nitrate depletion (Figs. 2.1A and C). Furthermore, flow cytometric analysis confirmed that *Synechococcus sp.* and small eukaryotic phytoplankton abundances peaked 1 to 2 days after nitrate depletion (data not shown).

Organic carbon and nitrogen - In all incubations, organic matter was initially partitioned mainly into the POM pool (ca. 78 to 100% of TOM), which was nitrogen-rich (C:N ~ 4 to 8) (Table 2.2). Accumulation of POM was concomitant with the development of phytoplankton blooms in each of the incubations (Figs. 2.2 and 2.3). Significantly more POC accumulated in the microplankton blooms (Figs. 2.2A-C) (mean = 300 ± 12.1 μmol L^{-1}) than in the nanoplankton bloom (Fig. 2.2D) (mean = 240 ± 11.4 μmol L^{-1}; *t*-test; $p < 0.05$). Similarly, significantly more PON accumulated in the microplankton blooms (Figs. 2.3A-C) (mean = 25.4 ± 0.43 μmol L^{-1}) than in the nanoplankton bloom (Fig. 2.3D) (mean = 20.1 ± 0.63 μmol L^{-1}; *t*-test; $p < 0.05$). Following nitrate depletion, the accumulated POM became enriched in carbon relative to nitrogen (Table 2.2) (C:N ~ 9 to 13.5). In three incubations, POC continued to increase by 44.0 to 131 μmol L^{-1} (mean = 83.7 ± 14.8 μmol L^{-1}) for one or more days (Figs. 2.2A, C, and D). Significant increases in PON were observed in only two of the incubations

Table 2.2. Partitioning of accumulated organic matter (OM) (as % of total accumulated organic matter) and elemental composition of the organic matter. Values designated "ns" indicate no significant accumulation. Values designated "*" indicate accumulation of either DOC or DON, but not both. Dashed lines indicate when nitrate went to depletion.

Inc. #	Day	% POC	%DOC	%PON	%DON	$C:N_{POM} \pm SD$	$C:N_{DOM} \pm SD$
1	0						
	1						
	2	100	0	100	0	4.33 ± 0.40	ns
	3	100	0	100	0	5.25 ± 0.11	ns
	4	60	40	74	26	8.39 ± 0.54	16.3 ± 8.65
	5	82	18	100	0	11.8 ± 0.57	*
	6	69	31	81	19	11.4 ± 0.30	22.5 ± 16.6
	7	74	26	80	20	11.4 ± 0.51	16.7 ± 10.0
2	0						
	1						
	2	88	12	100	0	6.87 ± 0.98	*
	3	79	21	78	21	5.21 ± 0.12	5.17 ± 5.19
	4	88	12	81	20	7.27 ± 0.25	4.18 ± 4.20
	5	71	29	85	15	12.8 ± 0.89	28.9 ± 15.3
	6	64	36	77	23	13.3 ± 0.83	24.7 ± 6.18
	7	60	40	74	26	13.5 ± 1.42	26.0 ± 6.94
	8	50	50	60	40	12.5 ± 0.34	18.4 ± 4.66
3	0						
	1						
	2	100	0	100	0	7.97 ± 1.70	ns
	3	100	0	100	0	6.12 ± 0.76	ns
	4	100	0	100	0	4.96 ± 0.53	ns
	5	100	0	100	0	5.13 ± 0.04	ns
	6	55	46	70	30	8.99 ± 1.15	17.2 ± 7.86
	7	65	35	100	0	9.28 ± 0.56	*
	8	65	35	100	0	10.5 ± 0.90	*
4	0						
	1						
	2	100	0	100	0	8.36 ± 7.46	ns
	3	100	0	100	0	4.29 ± 0.58	ns
	4	100	0	100	0	5.21 ± 0.25	ns
	5	100	0	100	0	5.30 ± 0.18	ns
	6	100	0	92	7	10.4 ± 0.76	*
	7	85	15	100	0	11.9 ± 0.33	*

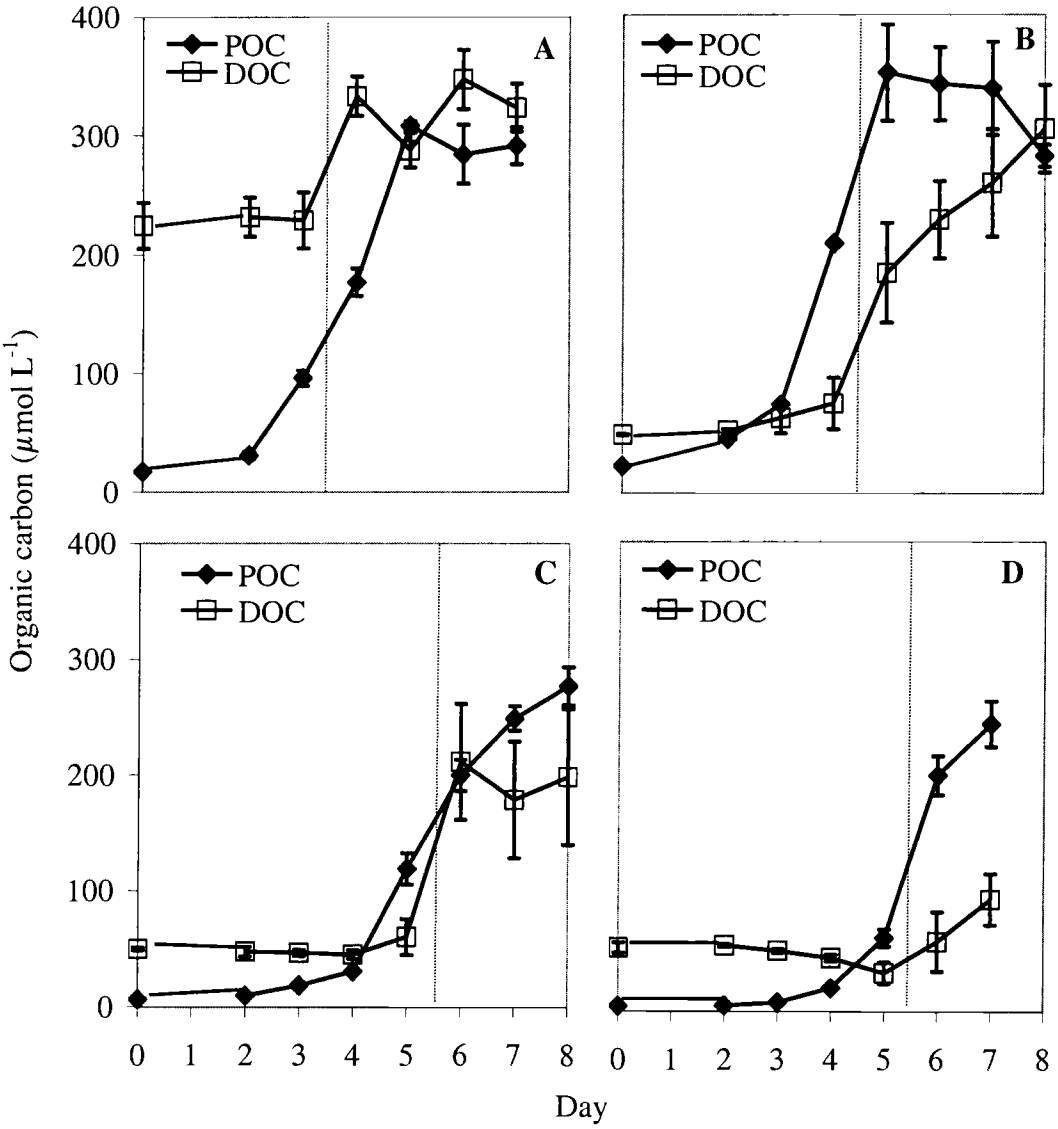

Figure 2.2. Temporal distribution and partitioning of organic carbon in deck incubations. Dashed lines indicate when nitrate went to depletion. Panels A-D correspond to incubation #1-4 respectively. Error bars represent standard deviations.

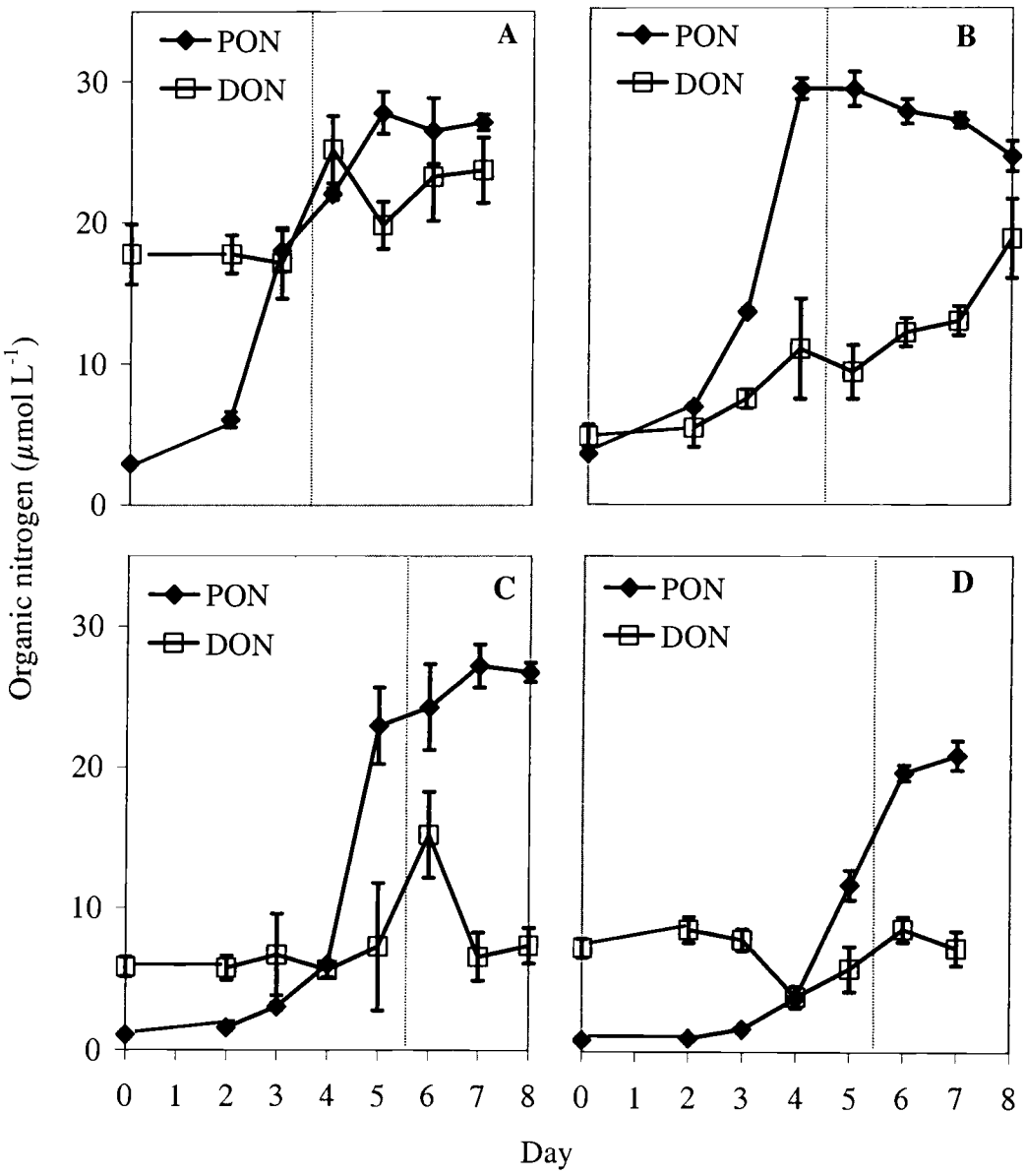

Figure 2.3. Temporal distribution and partitioning of organic nitrogen in deck incubations. Dashed lines indicate when nitrate went to depletion. Panels A-D correspond to incubation #1-4 respectively. Error bars represent standard deviations.

following nitrate depletion (Figs. 2.3A and C). Those increases amounted to 5.75 ± 1.56 μmol L^{-1} and 4.17 ± 3.11 μmol L^{-1}. In the other incubations, PON remained at a constant level (Fig. 2.3D) or decreased (Fig. 2.3B).

No DOM accumulated in nitrate replete conditions except in the second incubation when a relatively small (ca. 14.0 to 26.4 μmol L^{-1} DOC; 2.70 to 6.30 μmol L^{-1} DON), but statistically significant (*t*-test; $p < 0.05$) amount of nitrogen-rich (C:N ~ 4 to 5) DOM accumulated. Following nitrate depletion, carbon-rich DOM (C:N $\geq$ 16) rapidly accumulated over a one day period coinciding with nitrate depletion (Figs. 2.2 and 2.3; Table 2.2). Although the timing of the DOM increase was similar between incubations, the magnitude of the DOM accumulation was significantly less (*t*-test; $p < 0.05$) in the nanoplankton bloom (Figs. 2.2D and 2.3D)(41 μmol L^{-1} DOC and 2.8 μmol L^{-1} DON) than in the microplankton blooms (Figs. 2.2A-C and 2.3A-C) (109 to 161 μmol L^{-1} DOC and 6.3 to 9.4 μmol L^{-1} DON). Accumulated DOC comprised 18 to 50% of TOC produced in the microplankton blooms, but only 15% in the nanoplankton bloom. Approximately 42% of the initial accumulation of DOC and 80% of the initial accumulation of DON was drawn down the following day in the first incubation, but subsequently increased to near maximal values (Figs. 2.2A and 2.3A). In the other three incubations, DOC concentrations remained constant (Fig. 2.2C) or increased (Figs. 2.2B and D) following the initial accumulation. In two of the microplankton blooms, the initial DON buildup was transient as up to 92% was removed by the following day (Figs. 2.3A and C). During the transient peak, DON accounted for 15 to 30% of the accumulated TON pool in the microplankton blooms, but only 7% in the nanoplankton

bloom. Near the end of two of the incubations, DON subsequently increased by 3.60 to 8.67 μmol L^{-1} (mean = 6.14 ± 1.79 μmol L^{-1}) (Figs. 2.3A and B).

Calculated DIC assimilation – Based on measured concentrations of accumulated TOC and assuming Redfield stoichiometry of 6.63 mol carbon per mol nitrate, approximately 70 to 157% more carbon was fixed than can be supported by nitrate (Table 2.3). Most of the excess carbon fixation occurred immediately following depletion of nitrate. However, approximately 20 to 69% of the excess carbon fixation occurred one or more days after nitrate was initially depleted. In three of the incubations (#1, #3, #4), the excess carbon that was fixed in the days following nitrate depletion was measured as POM. The increase in the C:N content of the POM following nitrate depletion ranged from 9.90 to 25.8 in those incubations. In the other incubation (#2), the excess carbon that was fixed following nitrate depletion was measure as DOM.

DISCUSSION

As expected, diatom blooms developed in all four incubations, consistent with patterns of phytoplankton bloom formation in coastal upwelling systems (Chavez and Smith 1995). All of the diatom blooms appear to have been terminated by nitrate depletion, regardless of species composition. The ratio of nitrate to phosphate assimilation was similar in all blooms (13.1 to 15.6). However, the *Leptocylindrus minimus* bloom used about one-half as much silicate per unit nitrate as did the *Chaetoceros sp.* blooms, perhaps reflecting morphological or physiological differences between the species. Following nitrate depletion, chlorophyll in the < 3 μm size fraction remained elevated or

Table 2.3. Potential amount of CO_2 fixed, assuming C:N ratio of 6.625 mol CO_2 fixed per mol NO_3^- assimilated, in comparison to net accumulated TOC (μmol L^{-1}). Dashed lines indicate when nitrate went to depletion.

Inc. #	Day	NO_3^- (μmol L^{-1})	Potential CO_2 fixed (μmol L^{-1})	Avg CO_2 ± SD	Accumulated TOC ± SD
1	0	28.4	188	204 ± 13.6	
		31.8	211		
		32.2	213		
	2				20.9 ± 35.1
	3				83.4 ± 39.0
	4				269 ± 25.2
	5				354 ± 30.0
	6				390 ± 20.8
	7				373 ± 11.3
2	0	30.7	203	205 ± 1.79	
		31.1	206		
		31.1	206		
	2				25.8 ± 0.95
	3				65.8 ± 13.2
	4				213 ± 22.1
	5				464 ± 8.67
	6				499 ± 9.63
	7				526 ± 21.2
	8				527 ± 34.9
3	0	33.7	223	227 ± 3.11	
		34.5	229		
		34.6	229		
	2				2.10 ± 5.13
	3				8.70 ± 1.69
	4				18.9 ± 3.20
	5				125 ± 6.74
	6				351 ± 47.7
	7				370 ± 48.4
	8				418 ± 9.51
4	0	24.8	164	165 ± 2.11	
		25.2	167		
	2				3.36 ± 5.36
	3				0.75 ± 5.79
	4				6.94 ± 2.17
	5				36.6 ± 7.07
	6				201 ± 19.9
	7				281 ± 8.98

increased in two of the four incubations (Figs. 2.1A and C). Abundances of *Synechococcus* sp. and small eukaryotic phytoplankton, determined by flow cytometric analysis, also peaked 1 to 2 days after nitrate depletion, indicating that the cells might have been using regenerated nitrogen or DON (data not shown). There is precedent for phytoplankton growth using both ammonium and organic compounds in upwelling systems, particularly, although not exclusively, among cyanobacteria and nanoflagellates (Kokkinakis and Wheeler 1988; Probyn et al. 1990; Antia et al. 1991).

Partitioning of organic matter – Our nitrogen budgets show that nitrogen was completely conserved in the first two incubations. There was no significant difference (*t*-test; $p < 0.05$) in TN concentrations between the beginning and end of incubations #1 and #2. However, in the third and fourth incubations, ca. 83.7 ± 1.6% and 85.8 ± 1.2% of initial TN concentrations were found at the end of the experiments. Most of the nitrogen that was lost in the third incubation disappeared after the peak of the diatom bloom. Thus, incomplete recovery could have been due to undersampling of particulate matter that settled out and did not get completely mixed prior to sampling or exclusion of large diatom chains from POM analysis due to pre-filtration through a 202 μm mesh screen. Nearly all of the nitrogen that was lost (~ 4.5 μmol L^{-1}) in the fourth incubation disappeared between days 3 and 4, or prior to the peak of the phytoplankton bloom. Between those two days, DON also decreased by ~ 4 μmol L^{-1}. Although one could argue that the DON was being rapidly regenerated to ammonium, ammonium concentrations were near or below detection limits for the first five days of that incubation. Additionally, between days three and four when the DON decrease occurred,

bacterial abundances only increased slightly (from 0.97×10^6 to 1.15×10^6 cells ml^{-1}). Using those abundances and an average nitrogen content for coastal bacteria of 5.7 fg $cell^{-1}$ (Fukuda et al. 1998), bacterial particulate nitrogen would have increased from 0.40 $\pm$ 0.02 μmol L^{-1} to 0.47 $\pm$ 0.03 μmol L^{-1}. Thus it is unlikely that bacterial incorporation could account for the DON decrease. Unless phytoplankton uptake caused the DON drawdown, it seems likely that disappearance of the DON and TN from the fourth incubation could have been due to adhesion of the DON to the walls of the Cubitainers™.

Initial POM and DOM concentrations were similar to deep water concentrations seen by Hill and Wheeler (2002) off the Oregon coast, except in the first incubation where initial DOM concentrations were relatively high. The day after the first incubation began, a vertical profile from a more nearshore station showed elevated DOC (127 to 252 μmol L^{-1}) and DON (19 to 24 μmol L^{-1}) concentrations throughout the water column (P.A. Wheeler, unpublished data). This high DOM water could have been the source of the high initial DOM concentrations in the first incubation.

Newly produced, nitrogen-rich organic matter (C:N ~ 4 to 8) was partitioned mainly into the particulate pool (ca. 78 to 100% of total organic matter produced) as the phytoplankton blooms developed. Significant partitioning of organic matter into POM has been observed in high-latitude coastal waters (Wheeler et al. 1997; Carlson et al. 1998), although it has rarely been demonstrated in temperate coastal waters. Most studies in temperate systems have shown that bloom-derived organic matter accumulates mainly as DOM over the growing season (reviewed by Williams 1995; Carlson et al. 1998), and it has been suggested that major partitioning of organic matter as DOM is a

common phenomenon in marine waters (Sondergaard et al. 2000). However, during the peak of an episodic phytoplankton bloom off coastal Oregon, ca. 50 to 75% of accumulated organic matter was in particulate form in the upper 10 m of the water column (Hill 1999). Furthermore, as the bloom developed, DOM concentrations remained steady or decreased (Hill 1999). In a study of an embayment affected by coastal upwelling, Doval et al. (1997) found that ca. 60% and 70% of accumulated organic matter was in the form of POC and PON respectively. In our experiments, DOM only accumulated in one incubation prior to nitrate depletion. A small amount (~ 12 to 21% of accumulated organic matter) of nitrogen-rich DOM (C:N ~ 4 to 5) accumulated in the second incubation, dominated by *Chaetoceros* sp. Although grazing or viral lysis could have contributed to the DOM, phytoplankton excretion may also have been a source. It has been suggested that diffusive loss of small amounts of DOM may be a common characteristic of phytoplankton physiology, even among presumably healthy cells (Bjornsen 1988). However, the overall magnitude and ecological significance of such release is still subject to debate (Williams 1990), and our results suggest that DOM is a small fraction of primary production during exponential growth of coastal phytoplankton blooms.

Immediately following nitrate depletion, large amounts of carbon-rich DOM (C:N $\geq$ 16) appeared in the three microplankton blooms, with DOC representing 38 $\pm$ 8.5% of accumulated TOC and DON representing 24 $\pm$ 8% of accumulated TON. Although there are numerous mechanisms by which DOM can be produced, our results suggest that the DOM accumulation was related to the onset of nutrient limitation of the phytoplankton

blooms. Release of DOM by phytoplankton blooms that have become nutrient limited has been demonstrated in other marine systems (Lancelot 1983; Obernosterer and Herndl 1995). Phytoplankton cells often accumulate carbohydrates during exponential growth (Myklestad et al. 1989). At the transition between exponential and stationary growth phases or between nutrient replete and deplete conditions, phytoplankton are known to release large amounts of the accumulated dissolved carbohydrates, mainly as polysaccharides (Ittekkot et al. 1981; Myklestad et al. 1989; Biddanda and Benner 1997; Meon and Kirchman 2001). Empty diatom frustules were observed in fixed samples taken from several of our incubations following nitrate depletion, indicating that cell lysis or grazing contributed in part to the accumulation of DOC. However, increases in TOC concentrations following nitrate depletion imply that photosynthetic carbon fixation continued for several days. Nitrogen limitation affects the enzyme functioning of phytoplankton photosynthetic carbon metabolism and ultimately leads to a decreased ability for cells to dissipate light energy (Turpin 1991). It has been proposed that release of carbohydrates serves as an adaptive function in phytoplankton, in that it protects nutrient-limited cells growing in high light conditions from damage to their photosynthetic machinery by accommodating reductant supplied by light reactions (Wood and Van Valen 1990). Additionally, carbohydrate release has been implicated in aggregate formation (Passow et al. 1994), which in turn has been proposed as an adaptive mechanism by which diatom seed stocks are maintained in coastal upwelling regions (Smetacek 1985).

Although the DOM released by the microplankton blooms was carbon-rich, there was a considerable increase in DON. Release of DON by nitrogen-limited phytoplankton seems somewhat paradoxical, in that the cells are releasing a limiting resource and are also supplying substrate for bacterial competitors. However, DON release, accounting for 25 to 41% of nitrogen taken up by phytoplankton has been demonstrated in several different types of marine systems (Bronk et al. 1994). Obviously DON release due to cell lysis would be unavoidable, as would DON release due to grazing. Several studies have demonstrated DON accumulation during and after phytoplankton blooms and attributed a large portion of the accumulation to grazing (Bronk et al. 1998; Bronk and Ward 1999). However, numerous other studies have demonstrated release of varying quantities of amino acids by phytoplankton at the transition from exponential to stationary growth phase, and in the absence of grazing (Poulet and Martin-Jezequel 1983; Admiraal et al. 1986; Martin-Jezequel et al. 1988; Myklestad et al. 1989). In particular, Admiraal et al. (1986) observed a transient release of amino acids by the diatom *Thalassiosira excentrica* during the transition from exponential to stationary growth, suggesting that the release was due to a reduction in cell growth and metabolism.

In contrast to the incubations dominated by microplankton, relatively little DOC or DON accumulated in the nanoplankton bloom following nitrate depletion. One possible explanation for less DOM accumulation during the nanoplankton bloom is that there might have been a much tighter coupling between the DOM released and bacterial consumers than in the microplankton blooms. However, this scenario is not necessarily reflected in terms of bacterial abundance, which was relatively low compared to two of

the other incubations (data not shown). A more plausible explanation would be that there are differences in the size of intracellular DOM pools between the phytoplankton species (Martin-Jezequel et al. 1988), thus leading to differential release of DOM following nitrate limitation (Hellebust 1965). In terms of intracellular composition, Myklestad (1974) and Martin-Jezequel et al. (1988) have observed differences of several orders of magnitude in cellular carbohydrate and amino acid concentrations among different species of diatoms. These differences could explain, to some degree, differences in the percentage and magnitude of photoassimilated carbon and assimilated nitrogen excreted by different species of diatoms (Hellebust 1965; Admiraal et al. 1986).

Biogeochemical implications – Partitioning of primary production into either POM or DOM will have major consequences for the fate of that primary production by affecting export pathways. The results of our study and studies in other coastal systems suggest that POM can be a significant fraction of total primary production. Removal of POM from the euphotic zone can occur through sinking of the POM, vertical mixing of particles out of the euphotic zone, or horizontal transport due to advection. Elevated POM concentrations in bottom waters of the Oregon continental shelf suggests that there is a significant sinking flux of organic matter (Karp-Boss et al. unpublished data). In addition to the large accumulation of POM, we also saw significant accumulation of DOM following nitrate depletion of the phytoplankton. Phytoplankton bloom decay in Oregon coastal waters is frequently attributed to depletion of nitrate following cessation of upwelling (Wroblewski 1977; Corwith and Wheeler 2002). Excluding sampling conducted during a transient bloom, Hill and Wheeler (2002) found that roughly 63% of

net excess production of organic carbon accumulated as DOC during the upwelling season in coastal Oregon waters, suggesting that some DOM is released by primary producers, although it is unclear how much is released before cells sink out of the euphotic zone. Accumulation of large amounts of phytoplankton-derived DOC (reviewed by Williams 1995) and DON (Bronk et al. 1994) has been demonstrated in field studies conducted in other marine systems. Although it appears that the DON accumulation was transient in two of the four incubations in our study, little net change in accumulated DOC was observed. In a review of four different marine systems, Williams (1995) observed significant long-term (months) accumulation of carbon-rich DOM and attributed it to the inability of bacteria to remineralize the DOM due to nitrogen limitation. However, long term accumulation of DOC in surface waters off Oregon during the upwelling season is unlikely, given the dynamics of the alongshore and upwelling circulation (reviewed by Smith 1995). Investigations are currently underway to examine alongshore and cross-shelf variations in POM and DOM off Oregon in order to better understand which physical process influence the fate of organic material during the upwelling season.

Excess carbon fixation - Significantly more carbon was fixed than would be predicted based on Redfield stoichiometries of carbon consumption to nitrate assimilation by phytoplankton. Approximately 70 to 157% more carbon was fixed than would be predicted, with > 20 to 69% of the excess carbon fixation occurring after nitrate depletion. Photosynthetic carbon fixation above that predicted based on nitrate uptake has been demonstrated in situ (Sambrotto et al. 1993). In a laboratory study, Engel et al.

(2002) demonstrated excess carbon fixation by an experimental diatom bloom on the order of 72% more than predicted by nitrate assimilation, with nearly 100% of the excess fixed carbon going into the POM pool. Part of the POM pool (~40%) was determined to be TEP, a mucous-like substance that forms abiotically from dissolved carbohydrates (Passow 2000). Thus TEP is carbon-rich (C:N $\geq$ 26) (Engel and Passow 2001) and forms mainly after phytoplankton blooms become nutrient limited (Corzo et al. 2000; Engel et al. 2002). Because part of the TEP was measured as POM, rapid transformation of dissolved compounds into particulate material must have occurred. Following nitrate depletion, large amounts of carbon-rich DOM (C:N $\geq$ 16) accumulated in our incubations, and diatom aggregates loosely held together by a mucous-like substance were observed in the first three incubations, consistent with TEP production. Although some of the excess fixed carbon accumulated as DOC, it also accumulated as POC, suggesting either intracellular storage of the excess carbon or release as DOM and rapid transformation of the DOM into TEP-like particles. Aggregation of the cells or TEP-like material could be a mechanism by which excess fixed carbon is rapidly exported from the surface ocean. However, because little is known about the fate of sinking aggregates in situ, more work is needed to examine processes that might disrupt or breakdown aggregates prior to them sinking out of the euphotic zone (Smith et al. 1992).

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Response of bacteria to simulated phytoplankton blooms

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Response of bacteria to simulated phytoplankton blooms

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ABSTRACT

During exponential growth of phytoplankton in deck incubations conducted during the Oregon upwelling season, little or no dissolved organic matter (DOM) accumulation was observed and the percentage of daily primary production released extracellularly ranged from 7.13 to 19.2 % (mean = 12.7 ± 2.75). Bacterial abundances were analyzed via flow cytometry, and high nucleic acid (HNA) and low nucleic acid (LNA) bacteria were discriminated according to their side-scattering (related to cell size) and green fluorescence emission (related to nucleic acid content) properties. Abundances of HNA bacteria increased 2 to 3 fold in nitrate replete conditions, but the increases were relatively small ($< 1.0 \times 10^6$ cells ml^{-1}). Following nitrate depletion, carbon-rich (C:N $\geq$ 16) DOM accumulated in the incubations and the percentage of daily primary production released as dissolved organic carbon (DOC) ranged from 43.8 to 68.4 % (mean = 57.7 ± 10.7). Abundances and growth rates of HNA bacteria increased rapidly concomitant with the large DOM release, while little response was observed from cells with a LNA content. Although growth rates and abundances of HNA cells increased markedly, a net decrease in DOC was only observed in one incubation. Immediately after reaching maximum abundance, HNA bacterial abundance decreased rapidly while heterotrophic nanoflagellate abundances increased. These results indicate that both bottom- up and top- down controls may act to prevent bacterial degradation of phytoplankton DOM, thus allowing the short-term accumulation (several weeks) of carbon- rich DOM in the Oregon upwelling system.

INTRODUCTION

Recent studies have shown that autochthonously produced DOM is an important component of C and N budgets in upwelling systems (Álvarez-Salgado et al. 2001; Hill and Wheeler 2002). Because DOM is a component of primary production, export estimates should take it into account. However, export pathways for DOM are less readily understood than for POM. Phytoplankton-derived DOM is a chemically complex pool, which affects its biological availability and influences export pathways. Some DOM produced by phytoplankton is rapidly (hours to days) respired, and DOM that is assimilated by microbial communities can largely be ignored in export estimates due to the fact that the microbial food web contributes little to the sinking flux of POM (Eppley and Peterson 1979). Export of DOM not affected by microbial activity must then be through physical mechanisms such as vertical mixing or horizontal advection. Incorporation of DOM into rapidly sinking aggregates through abiotic processes is an additional export pathway that has recently been recognized (Passow 2000).

During a time-series study at a coastal Oregon site influenced by upwelling, Hill and Wheeler (2002) found that roughly 63 % of the organic carbon produced in excess of deep water concentrations was as DOC, suggesting a potentially pronounced role for DOM in the Oregon upwelling system. There are two main sources of DOM to coastal Oregon waters; phytoplankton exudation and the Columbia River (Hill and Wheeler 2002). The Columbia River has a distinct salinity signature of < 32 psu and can easily be distinguished from upwelled water. Hill and Wheeler (2002) determined that most of the

DOM produced in excess of deep- water concentrations that was measured in coastal waters was from decaying phytoplankton blooms.

Upwelling events off Oregon typically last up to two weeks and are terminated when winds reverse to downwelling favorable, which breaks down stratification of surface waters (Smith 1995). Although the physical dynamics (i.e. advection, vertical mixing) of the Oregon upwelling system likely prevent a significant seasonal accumulation of C-rich DOM, it is clear that phytoplankton derived DOM can be a quantitatively important component of C and N budgets over relatively short time scales (Wetz and Wheeler in press), especially considering that phytoplankton blooms are frequently terminated by nitrate limitation (Wroblewski 1977; Corwith and Wheeler 2002). The goal of this work was to examine the response of bacteria to simulated phytoplankton blooms and to interpret the response in terms of bottom- up and top- down controls of accumulation of phytoplankton-derived DOM in the Oregon upwelling system. We hypothesized that N limitation of the bacteria would be the primary cause for accumulation of phytoplankton-derived DOM (e.g. Williams 1995). Additionally, given the now widely recognized role of heterotrophic nanoflagellates as bacterivores (Sherr and Sherr 1994), we also examined the response of heterotrophic nanoflagellates and evaluated their role as a potential top-down control on bacterial biomass.

METHODS

A detailed description of the experimental design can be found in Wetz and Wheeler (in press). Briefly, experiments were run over a three-week period in August 2001 during

the peak of the upwelling season. Water samples for two incubations (#1 and #2) were collected from mid-shelf sites off northern Oregon (45.00 N, 124.02 W) and water samples for the other two incubations (#3 and #4) were collected from mid-shelf sites off central Oregon (44.13 N, 124.28 W and 44.06 N, 124.33 W respectively).

For each incubation, triplicate 20-L HDPE Cubitainers™ were filled with 1 L of surface water inoculum and 19 L of water from below the mixed layer. Seawater was pumped directly into the Cubitainers™ from depth using a towed sled with a pump attached. Prior to being filled, Cubitainers™ were vigorously rinsed with 10 % HCl followed by multiple rinses with deionized water. Cubitainers™ were held in a deck incubator at *in situ* surface water temperatures averaging 13.5^0 C. The incubator was covered with one layer of neutral density mesh screen that reduced light intensities to approximately 50 % of surface intensities. There were approximately 14 hours of light and 10 hours of darkness daily during the cruise and daily integrated PAR values at the surface ranged from 15.0 to 52.0 mol quanta m^{-2} d^{-1} (mean = 31.6 ± 12.0 mol quanta m^{-2} d^{-1}). Moderate mixing of samples occurred due to the motion of the boat, and the samples were vigorously mixed manually once or twice daily. Cubitainers™ were sampled daily in the early morning (1400 to 1700 GMT). Incubations lasted 7 to 8 days.

Biological analyses - Flow cytometric analysis was done using a Becton-Dickinson FACSCalibur flow cytometer with a 15 mw laser at 488 nm following the protocol of Marie et al. (1997). Briefly, samples were preserved with freshly made 2 % paraformaldehyde, quick-frozen in liquid nitrogen, and stored at -80^0 C until flow cytometric analysis in the lab. For each sample, 250 μl of 0.2 μm filtered deionized

water was pipetted into a cytometer tube, followed by 45 μl of 0.2 μm filtered potassium citrate. Next, 250 μl of freshly thawed sample was pipetted into the cytometer tube, followed by 5 μl of the nucleic acid stain, SYBR Green I (Molecular Probes, Eugene OR). Finally, after brief vortexing and storage in the dark for ca. 10 minutes, 25 μl of 1.0 μm calibrated beads of known abundance were pipetted into the cytometer tube and the sample was briefly vortexed again. Samples were analyzed at low flow rates (ca. 10-12 μl minute^{-1}), and bacteria were discriminated according to their side-scattering (related to cell size) and green fluorescence emission (measured at 530 nm) properties. Analyses were performed on a logarithmic scale, and gates (windows) were drawn around HNA LNA groups of cells to delimit them. When the coefficient of variation for the triplicate bacterial abundance measurements was > 15 %, a sample value was removed if it was greater than one standard deviation away from the mean of the triplicates. Seven values out of ninety measurements total were excluded. Average coefficient of variation for all replicates was 7.85 ± 4.52 %. Growth rates were calculated assuming exponential growth, as shown in Eq. (1).

$$\mu = (\ln (N/N_o))/t \qquad (1)$$

Samples for heterotrophic nanoflagellate abundance were collected in 250 ml high-density polyethylene bottles predispensed with 150 μl of alkaline Lugols solution. Immediately after collection, 7.5 ml of borate buffered formalin and 300 μl of 3 % sodium thiosulfate was added. After storage in the refrigerator for ca. 12 h, all but 2 ml of a 10 to 50 ml sample was vacuum filtered (<5 mm Hg) onto a 0.8 μm black membrane filter. Then, 50 μl of DAPI was added to the remaining 2 ml of sample, and the sample

was allowed to sit for 7 minutes in the dark. Finally, the remaining 2 ml of sample was filtered onto the black membrane filter. Heterotrophic nanoflagellates were counted at 400x magnification using a ZEISS epifluorescent microscope and were distinguished from phototrophic nanoflagellates by alternating between UV and blue light excitation. A minimum of 20 counting grids or 200 cells were counted for each sample. The potential grazing impact of heterotrophic nanoflagellates on bacteria was assessed by comparing the amount of heterotrophic nanoflagellate carbon biomass and respiration to the decreases in bacterial abundance and carbon biomass that occurred at the end of the first three incubations. Heterotrophic nanoflagellate carbon biomass was calculated using observed abundances and the equation of Menden-Deuer and Lessard (2000) relating protist biovolume to cellular carbon content. Growth efficiency of the flagellates was assumed to be 30%, which is an average of values reported in the literature (see Table 7.5 in Caron and Goldman 1990). Bacterial carbon biomass was calculated using observed abundances and assuming a carbon content for coastal bacteria of 30 fg $cell^{-1}$ (Fukuda et al. 1998).

Chemical analyses - Nutrient samples were collected in acid-washed 30 ml high-density polyethylene bottles and immediately frozen at – 30^0 C. Samples were analyzed on a Technicon AA-II according to standard wet chemical methods of Gordon et al. (1995). Total organic carbon (TOC) samples were collected in borosilicate vials with Teflon cap liners. Each vial contained approximately 5 ml of seawater that was preserved with 50 μl of 90 % phosphoric acid. Samples were stored at room temperature until being processed 3 months later. Samples were analyzed over a three day period

using the High Temperature Catalytic Combustion method on a Shimadzu TOC-5000A analyzer. Particulate organic carbon (POC) was determined from material collected on precombusted GF/F filters. Water samples were pre-filtered through a 202 μm mesh screen to remove zooplankton. Then, 500 or 1000 ml of the filtered water was vacuum filtered (< 200 mm Hg) onto precombusted GF/F filters. After filtration, samples were stored in glass Vacutainers^{TM} and immediately frozen at - 30^0 C until laboratory analysis. Samples were processed within 2 months of collection. Filters were fumed with hydrochloric acid to remove inorganic carbon and dried, followed by analysis using a Control Equipment Corp. 440HA CHN elemental analyzer calibrated with acetanilide. Dissolved organic carbon was determined by subtracting POC values from TOC values, as shown in Eq. (2).

$$DOC = TOC - POC \qquad (2)$$

The standard deviation for DOC was calculated by propagation of error using standard deviations for TOC and POC (Bevington 1969). More detailed descriptions of all the chemical methods used can be found in Wetz and Wheeler (in press).

RESULTS

Phytoplankton bloom development and organic matter production - Phytoplankton blooms developed in all four incubations and chlorophyll *a* peaked in 4 to 6 days, with maximum chlorophyll *a* concentrations ranging from 23 to 41 μg Chl *a* L^{-1}. In the first three incubations, blooms were composed primarily of the diatom *Chaetoceros sp.*, while in the fourth incubation the bloom was composed primarily of the smaller diatom

Leptocylindrus minimus. All of the blooms were terminated due to nitrate depletion. Initially, organic matter accumulated mainly as POM (ca. 78 to 100 % of total organic matter). No DOM accumulated in nitrate replete conditions except in the second incubation where a relatively small amount (ca. 14.0 to 26.4 μmol L^{-1} DOC; 2.70 to 6.30 μmol L^{-1} DON) of nitrogen-rich (C:N ~ 4 to 5) DOM accumulated. Using an average carbon content for coastal bacteria of 30 fg $cell^{-1}$ (Fukuda et al. 1998) and an average growth efficiency for coastal bacteria of 27 % (del Giorgio and Cole 2000), we estimate that the total amount of DOC needed to account for bacterial growth and metabolism at the beginning of the incubations ranged from 3.62 to 7.34 μmol L^{-1} d^{-1} (mean = 5.05 ± 1.61). By the time nitrate went to depletion, but prior to the large accumulation of DOC, the amount of DOC needed for bacterial growth ranged from 10.3 to 16.3 μmol L^{-1} d^{-1} (mean = 12.7 ± 2.75). By adding the DOC demand of the bacteria to the amount of carbon fixed (as TOC; see Wetz and Wheeler in press) and dividing the DOC estimate by that term, we estimate that the percentage of daily primary production released extracellularly (PER) by the phytoplankton during exponential growth ranged from 7.1 to 19.2 % (mean = 12.4 ± 12.0).

Immediately following nitrate depletion, large amounts of carbon-rich DOM (C:N ≥ 16) accumulated over a period of one day. In blooms dominated by *Chaetoceros sp.*, 109 to 161 μmol L^{-1} DOC and 6.3 to 9.4 μmol L^{-1} DON accumulated, and in the *L. minimus* dominated bloom, 41 μmol L^{-1} DOC and 2.8 μmol L^{-1} DON accumulated. Using previously stated assumptions concerning bacterial C content and growth efficiency, PER would range from 43.8 to 68.4 % (mean = 57.7 ± 10.7) immediately following nitrate

depletion. Approximately 42 % of the initial accumulation of DOC and 80 % of the initial accumulation of DON was drawn down the following day in the first incubation, but subsequently increased to near maximal values. In the other three incubations, DOC concentrations remained constant or increased following the initial accumulation. In the first and third incubations, the initial DON buildup was transient as 80 % and 92 %, respectively, were removed by the following day. More complete details concerning the phytoplankton bloom development and chemical measurements can be found in Wetz and Wheeler (in press).

Bacteria abundance and DNA content - During exponential growth of the phytoplankton, relatively little change in bacterial abundance occurred (Figure 3.1). Abundances of HNA cells increased by 2 to 3- fold from the beginning of the experiments until nitrate was depleted (Figure 3.2), and their growth rates ranged from 0.19 to 0.33 d^{-1} (mean = 0.24 ± 0.07) (Table 3.1). Abundances of LNA cells increased slightly during the development of the phytoplankton blooms, but the increases were $\leq 0.16 \times 10^6$ cells ml^{-1} in all four incubations (Figure 3.2). Growth rates of LNA cells ranged from 0.08 to 0.20 d^{-1} (mean = 0.12 ± 0.06) during that time (Table 3.1).

Immediately after nitrate was depleted and the phytoplankton blooms began to decline, bacterial abundances increased markedly (Figure 3.1). This increase occurred concomitant with the DOM release from the decaying phytoplankton blooms. Abundances of HNA cells increased 2 to 9- fold during the DOM buildup, with maximum abundances ranging from 3.67 to 12.3 x 10^6 cells ml^{-1} (Figure 3.2). Growth rates of HNA cells increased noticeably as well, ranging from 0.38 to 1.11 d^{-1} (mean =

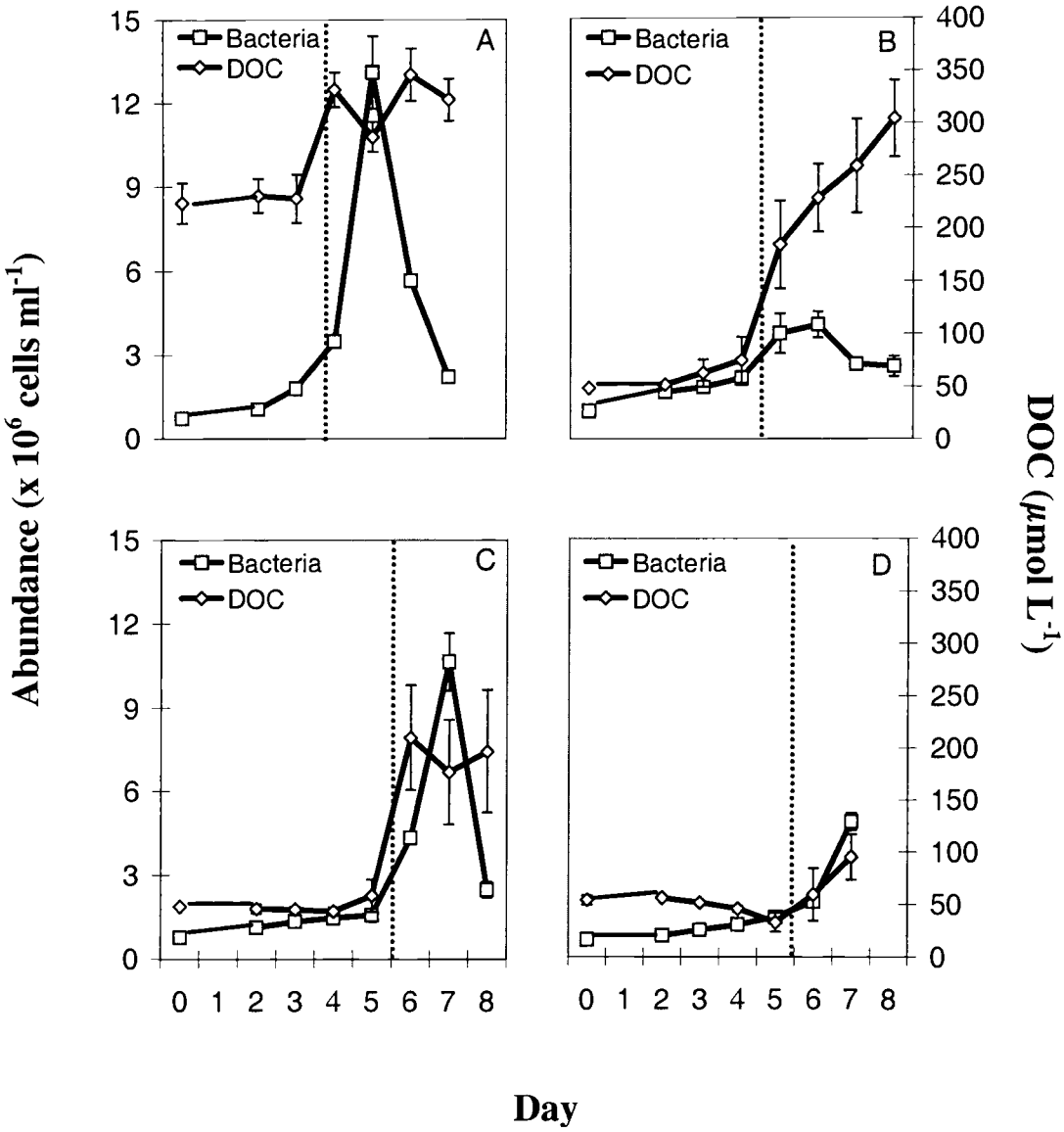

Figure 3.1. Temporal distribution of dissolved organic carbon (μmol L^{-1}) and bacteria (x 10^6 cells ml^{-1}) in deck incubations. Dashed lines indicate when nitrate went to depletion. Panels A-D correspond to incubation #1-4 respectively. Error bars represent standard deviations.

Table 3.1. Growth rates (d^{-1}) of HNA and LNA bacteria before and after nitrate limitation of phytoplankton blooms. Growth rates were calculated assuming exponential growth. Note that μ for low DNA bacteria from days 0 to 5 in incubation # 3 has no SD due to only one replicate being available.

		HNA	LNA
Inc. #	**Days**	**μ (d^{-1}) ± SD**	**μ (d^{-1}) ± SD**
1	0 to 3	0.33 ± 0.02	0.20 ± 0.04
	3 to 5	1.11 ± 0.07	0.46 ± 0.05
2	0 to 4	0.20 ± 0.06	0.08 ± 0.04
	4 to 6	0.38 ± 0.15	negative
3	0 to 5	0.19 ± 0.00	0.11
	5 to 7	1.11 ± 0.13	0.06 ± 0.09
4	0 to 5	0.21 ± 0.01	0.08 ± 0.02
	5 to 7	0.69 ± 0.06	0.05 ± 0.00

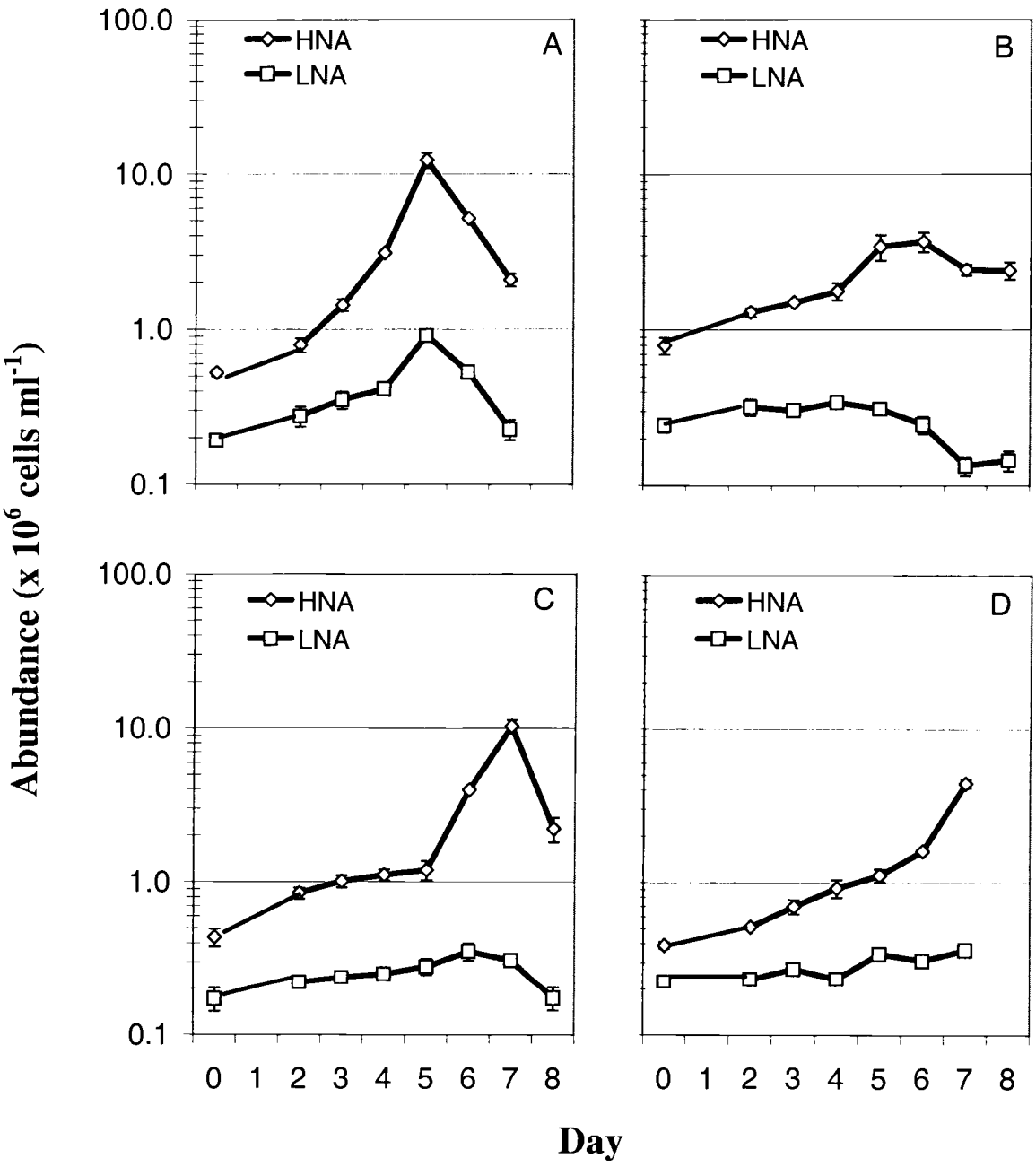

Figure 3.2. Abundance of HNA and LNA bacteria in deck incubations (x 10^6 cells ml^{-1}). Panels A-D correspond to incubation #1-4 respectively. Error bars represent standard deviations.

0.81 ± 0.32) (Table 3.1). In contrast to the response by the HNA bacteria, abundances of LNA bacteria increased significantly (t-test; $p < 0.05$) in only one incubation (#1) during the period of DOM buildup (Figure 3.2). The growth rate of LNA bacteria in that incubation was 0.46 d^{-1} (Table 3.1). In the other incubations, abundances of LNA bacteria remained constant (#3 and #4) or decreased (#2) (Figure 3.2). Similarly, growth rates of LNA cells in incubations #3 and #4 were 0.06 d^{-1} and 0.05 d^{-1} respectively, and growth rates in incubation #2 were negative (Table 3.1). After several days of rapid growth, abundances of HNA bacteria in the first three incubations peaked and then decreased considerably over the remaining 1 to 2 days of the incubations (Figure 3.2). It is unclear whether the bacteria had reached maximum abundance in the fourth incubation due to termination of the experiment on day 7.

Heterotrophic nanoflagellate abundance - All heterotrophic flagellates enumerated were smaller than 12 μm in diameter. However, the contribution of larger mixotrophic dinoflagellates and naked flagellates could not be quantified, but autofluorescing cells of both groups were present. Abundances of heterotrophic nanoflagellates were low at the start of the incubations, ranging from 100 to 500 cells ml^{-1} (mean = 300 ± 100) (Table 3.2). Between the beginning of the incubations and the end of exponential growth of the phytoplankton, flagellate abundance increased by 2.5 to 7- fold (Table 3.2). The largest absolute increases in flagellate abundance occurred following nitrate depletion, and maximum abundances ranged from 3,000 to 13,300 cells ml^{-1} (mean = 5,900 ± 4,600).

Table 3.2. Abundances (cells ml^{-1}) of heterotrophic nanoflagellates in deck incubations.

Inc. #	Day	HNAN Abundance (cells ml^{-1}) ± SD
1	0	100 ± 0
	3	300 ± 100
	6	3300 ± 1000
2	0	300 ± 100
	3	1500 ± 200
	6	4000 ± 600
3	0	500 ± 100
	5	3300 ± 0
	8	13300 ± 1400
4	0	200 ± 0
	5	1200 ± 100
	7	3000 ± 500

DISCUSSION

During growth of a phytoplankton bloom off coastal Oregon, ca. 50 to 75 % of accumulated organic matter was in particulate form in the upper 10 m of the water column and DOM concentrations remained steady or decreased (Hill 1999). Similarly, DOM concentrations remained constant in our incubations prior to nitrate depletion, with the exception of a small decrease in the fourth incubation that was attributed to adsorption of DOM to the container walls (Wetz and Wheeler in press).

Although DOM concentrations generally remained constant during exponential growth of the phytoplankton, small increases in the abundance of HNA bacteria were observed. The lack of decrease in the initial C-rich DOM (C:N = 7.5 to 12.7) coupled with bacterial growth indicates that the background or deep-water DOM was relatively refractory material (Carlson 2002; Hill and Wheeler 2002). Thus, if bacterial growth was not supported by the deep-water DOM, bacteria must have been using DOM excreted either by the growing phytoplankton or from grazers. Diffusive loss of small amounts of DOM may be a common characteristic of phytoplankton physiology, even among presumably healthy cells (Bjørnsen 1988). However, the ecological significance of such DOM release by healthy phytoplankton is still subject to considerable debate (Williams 1990). Given that little or no DOM accumulated prior to nitrate depletion and that PER only averaged 12.4 %, our results suggest that DOM is a small fraction of primary production during exponential growth of phytoplankton in upwelling blooms. Furthermore, the estimated PER should be considered a maximum estimate if the bacteria were meeting some of their carbon demand from DOM excreted by grazers, although the

observed heterotrophic nanoflagellate abundances during the early stages of the incubations were relatively small.

Immediately following nitrate depletion, large amounts of carbon-rich DOM (C:N ≥ 16) appeared in the first three incubations and a smaller amount appeared in the fourth incubation (Wetz and Wheeler in press). Additionally, PER increased to 57.7 ± 10.7 %, consistent with studies in other marine systems and laboratory studies showing enhanced release of DOM by nutrient limited phytoplankton (reviewed by Carlson 2002). Phytoplankton often accumulate carbohydrate during exponential growth which is later released in large quantities at the transition between exponential and stationary growth phases or between nutrient replete and deplete conditions (Ittekkot et al. 1981; Myklestad et al. 1989; Biddanda and Benner 1997). Although the DOM released was carbon-rich, there was a considerable increase in DON as well. Several studies have demonstrated release of varying quantities of amino acids by phytoplankton at the transition from exponential to stationary growth phase (Poulet and Martin-Jézéquel 1983; Admiraal et al. 1986; Myklestad et al. 1989).

Abundances and growth rates of HNA bacteria increased rapidly concomitant with the large DOM release. In contrast, little response was observed from cells with a LNA content. HNA bacteria are generally assumed to correspond to the metabolically active fraction of marine bacteria, while LNA bacteria are thought to be dead or inactive cells (Gasol et al. 1999; Gasol and del Giorgio 2000). This assumption has recently been questioned by Zubkov et al. (2001) who found that in oligotrophic waters of the Celtic Sea, LNA cells actually had the highest cell-specific growth rates. In terms of metabolic

activity though, field studies in a variety of systems have clearly demonstrated that HNA cells account for most of the bacterial production and have higher cell specific activities than LNA cells (Lebaron et al. 2001; Vaqué et al. 2001; Zubkov et al. 2001). This trend has also been observed for bacterioplankton communities in coastal Oregon waters (K. Longnecker et al. submitted).

Although growth rates and abundances of HNA cells increased markedly in response to the DOM buildup, a decrease in the phytoplankton- derived DOC was only observed in one incubation. In the first incubation, a net decrease of approximately 45 μmol L^{-1} DOC and 5.3 μmol L^{-1} DON occurred as bacterial abundances peaked (Figure 3.1A). Assuming a cell content for coastal bacteria of 30 fg C and 5.7 fg N (Fukuda et al. 1998) as well as a carbon growth yield of 27 % (del Giorgio and Cole 2000), we estimate that bacterial uptake could account for all of the DOC usage and roughly 73 % of DON usage. The lack of change in DOM concentrations in the other incubations, despite enhanced bacterial growth, could suggest that the transient bacterial response was stimulated by the relatively labile fraction of DOM (i.e. sugars, amino acids, soluble proteins) that was turned over rapidly enough to be missed in our bulk DOM measurements (Chen and Wangersky 1996). We would need to characterize the phytoplankton DOM on a finer level and also get estimates of turnover times of specific DOM pools in order to better elucidate which components of the DOM pool prompted the bacterial response.

Immediately after reaching maximum abundance, HNA bacterial abundance declined to levels approaching initial abundances. Given the large increases in hetertrophic nanoflagellate abundance, bacterivory must have contributed in part to the decline.

Bacterivory is now widely recognized as having a key role in C and N cycling in marine environments (Sherr and Sherr 1994). Laboratory (González et al. 1993) and field studies (Sherr et al. 1992; del Giorgio et al. 1996) have demonstrated feeding selectivity by phagotrophic flagellates, which often show preferences for actively dividing cells (i.e. HNA bacteria) over dead or dormant cells (del Giorgio et al. 1996; Lebaron et al. 1999; Vaqué et al. 2001). Assuming that the flagellates were selectively feeding on the high DNA bacteria, we estimate that 27 % of the decrease in the first incubation was due to grazing by flagellates, and > 100 % of the decrease in bacterial abundance in the second and third incubations was due to grazing by flagellates. Variability in these estimates is high due to the assumed HNAN growth efficiencies and variability in the replicate flagellate and HNA bacterial abundances. Given that in two of the incubations, more flagellate carbon was present than would be predicted by the decrease in bacterial abundance, it is likely that the flagellates were also feeding on small phytoplankton and not just bacteria (e.g. Caron et al. 1992). However, flagellate grazing must have contributed in part to the decline in bacterial abundance.

It is inherently difficult to extrapolate the results of laboratory studies to field situations. However, as in our case where we were interested in observing organic matter production by coastal phytoplankton, the incubations proved to be advantageous. The physical dynamics of the Oregon upwelling system make studying *in situ* blooms from initiation to decay difficult. Advection and vertical mixing leave Langrangian drogue studies open to interpretation, and those types of studies would additionally require spending more time at sea, with no guarantee of observing a bloom. Our studies allowed

us to repeatedly examine organic matter production throughout the entire course of diatom blooms. Although the impact of zooplankton grazing could have been decreased due to incubation of the water, there is considerable evidence that most phytoplankton blooms off Oregon are terminated due to nitrogen limitation, not zooplankton grazing (Wroblewski 1977; Corwith and Wheeler 2002; Peterson et al. 2002).

Mesocosm studies in other systems have shown accumulation of similar concentrations of DOC and DON following nutrient limitation of diatom blooms (Norrman et al. 1995; Sondergaard et al. 2000). Maximum *in situ* DOC concentrations measured in excess of deep-water values off Oregon are roughly 100 μmol L^{-1} less than those measured in the incubations (Hill and Wheeler 2002). However, there is no *a priori* reason to expect a difference in the magnitude of DOM release observed in the incubations and *in situ*, given that phytoplankton stocks (in terms of chl *a*) observed in the incubations have also been observed *in situ* (e.g. Small and Menzies 1981; Neuer and Cowles 1994). Because the DOM that accumulated in our experiments (C:N $\geq$ 16) was probably dominated by carbohydrates, our experiments likely did not last long enough for noticeable bacterial enzymatic breakdown of the material to occur. That would account for some of the discrepancy between our estimates of accumulated DOC and those observed *in situ*. However, it is questionable as to how much DOC would degrade with time, given the rapid decrease in bacterial abundance and the apparent resistance of some phytoplankton DOM to degradation (Fry et al. 1996).

Studies during the upwelling season on the Oregon shelf (Sherr et al. 2001) and Iberian shelf (Morán et al. 2002) showed that there was no significant correlation

between rates of bacterial production and DOC concentration, with Sherr and Sherr (1996) and Morán et al. (2002) suggesting that the activities of phytoplankton and bacteria can be temporally uncoupled. Our results show a transient response to phytoplankton DOC, with an apparent strong coupling between phytoplankton DOM and bacteria during exponential growth of the phytoplankton. However, top- down and bottom- up controls acted to prevent bacterial abundances from remaining elevated. Thus bacterial abundances (and presumably community activity) became uncoupled from phytoplankton produced DOM as the bloom decayed. As previously discussed, bacterivory is one obvious control. Viral lysing is another possible control that could have caused the rapid decrease in bacterial abundance (e.g. Fuhrman and Noble 1995), but it was not assessed in this study. Additionally, the role of nitrogen limitation of the bacteria must be considered (Williams 1995). Because we do not know whether the measured DON was associated with the DOC (i.e. as amino sugars) or if it was part of a separate N rich DOM pool, it is difficult to say whether it was bioavailable over relevant time scales (i.e. days to weeks). In addition, continuous production of ammonium produced through grazing activities could partially alleviate the N limitation. However, development of a secondary phytoplankton bloom based on recycled nutrients (i.e. ammonium or DON) in the incubations presumably increased competition for the already limited nitrogen supply (M.S. Wetz, unpublished data).

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CONCLUSIONS

Because of the physical dynamics associated with coastal upwelling systems, few studies have examined the partitioning of organic matter in upwelling systems, despite the fact that they play a key role in carbon and nitrogen budgets in the ocean. The deck incubations proved to be advantageous by allowing us to repeatedly examine organic matter production throughout the entire course of diatom blooms. In hindsight, probably the biggest drawback of the incubations is that they likely minimized the impact of grazing by migratory zooplankton. However, even this is probably not a major concern, considering that most phytoplankton blooms off Oregon are terminated due to nitrogen limitation, not zooplankton grazing (Wroblewski 1977; Corwith and Wheeler 2002; Peterson et al. 2002).

Partitioning of primary production into either POM or DOM will have major consequences for the fate of that primary production by affecting export pathways. During exponential growth of the phytoplankton in our deck incubations, $\geq$ 78% of total accumulated organic matter was as POM and the percentage of daily primary production released extracellularly averaged 12.4 % $\pm$ 12.0 %. This suggests that DOM is a small fraction of primary production during exponential growth of coastal phytoplankton blooms. Elevated POM concentrations in bottom waters of the Oregon continental shelf suggests that there is a significant sinking flux of organic matter (Karp-Boss et al. unpublished data). We also saw significant accumulation of phytoplankton- derived DOM, but only following nitrate depletion of the phytoplankton. Immediately after nitrate depletion, the percentage of daily primary production released as DOC increased

to 57.7 % ± 10.7 %. It is likely that the DOM pool was a major fate for the "excess carbon" fixation that we observed, which was 70 to 157% more than can be supported by nitrate assuming Redfield stoichiometry.

Although maximum *in situ* DOC concentrations measured in excess of deep-water values off Oregon are roughly 50 to 100 μmol L^{-1} less than those measured in our incubations, there is no *a priori* reason to expect a difference in the magnitude of DOM release observed in the incubations and *in situ*, given that phytoplankton stocks (in terms of chl *a*) observed in the incubations have been observed *in situ* (e.g. Small and Menzies 1981; Neuer and Cowles 1994). The findings of Hill and Wheeler (2002) that roughly 63% of organic carbon produced in excess of deep water concentrations accumulated as DOC during the upwelling season suggests that some DOM is released by primary producers.

The accumulation of phytoplankton- derived DOM and excess carbon fixation, along with the differences in magnitude of accumulated DOM in the incubations vs. *in situ*, points to the need for several future investigations. There are two main issues that would be logical follow-ups to these experiments. They include a need for further study of the residence time of bloom-forming phytoplankton in the euphotic zone relative to nitrate supply during upwelling events and examination of degradation rates of phytoplankton-derived DOM and controls on the degradation of that material.

It is clear that the large DOM release observed in our incubations was facilitated by high light intensities and nutrient deplete conditions, as has been demonstrated in other systems (reviewed by Wood and Van Valen 1990). Thus, rapid removal of cells from the

euphotic zone prior to nutrient depletion could act to prevent the large extracellular release of DOM by slowing down their photosynthetic activities. Individual diatom cells sink much too slowly (< 2 m day^{-1}) to sink out of the euphotic zone in the timeframe observed for DOM release in our incubations (Smayda and Boleyn 1966). However, specific mechanisms such as aggregation have been identified as causing phytoplankton to sink rapidly (> 100 m d^{-1}) (Alldredge and Gotschalk 1989). Although aggregation has been studied intensely in the past decade, there are discrepancies in the literature concerning the timing of aggregation events in terms of bloom stage. Aggregation is frequently coupled with DOM production (e.g. TEP), but the problem revolves around whether the small amounts of DOM released during exponential growth of phytoplankton is enough to lead to mass aggregation prior to nutrient depletion. Alldredge et al. (1995) demonstrated that the timing of aggregation events was not related to nutrient concentrations, while incubation studies by Corzo et al. (2000) and Engel et al. (2002) suggest that aggregation occurs mainly following nutrient depletion. Clarification of the timing of aggregation events and also examination of species- specific aggregation will help to better understand the likelihood of nutrient- limited DOM release *in situ*.

The second issue that merits further study is, in simplest terms possible, the role of phytoplankton- derived DOM in lower trophic level food web dynamics. Our results show a transient bacterial response to the phytoplankton DOM. However, the lack of change in DOM concentrations despite enhanced bacterial activity suggests that the transient bacterial response was stimulated by a relatively labile fraction of the DOM (i.e. sugars, amino acids, soluble proteins) that was turned over rapidly enough to be missed in

our bulk DOM measurements (Chen and Wangersky 1996). That would imply that the bulk of the material was relatively resistant to degradation over the timeframe of our incubations (i.e. days). This is important because accumulation of resistant material means that the physical dynamics of the system will control export of the material from the system . Furthermore, it also means that nitrate assimilation may underestimate gross primary production, as per our incubations. Before any firm conclusions can be drawn however, a characterization of the phytoplankton DOM on a finer level is needed, as are estimates of turnover times of the specific DOM pools. Studies are also needed to examine other bottom- up and top- down controls on bacterial biomass and activity that act to prevent degradation of the DOM. We found evidence for both flagellate grazing and nitrogen limitation as controls, but one must also consider the recently discovered role of viral lysing as an additional control on bacterial biomass (Fuhrman and Noble 1995).

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APPENDIX

Appendix: Data from August 2001 deck incubations

The following pages show all of the data from the four incubations conducted on the August 2001 COAST cruise. Blanks in the table indicate that either no data was available or that a data point was considered an outlier and excluded from analysis. Note that abundances of large eukaryotic phytoplankton in the first incubation have been excluded due to problems with the calibration beads used in the flow cytometric analysis of those samples.

Incubation Number	Day	Replicate	TOC (μM)	POC (μM)	DOC (μM)	TN (μM)	PON (μM)	DON (μM)
1	0	a	260.26	17.35	242.91	53.11	3.00	20.21
		b	242.81	17.42	225.40	53.05	2.89	16.72
		c	222.10	16.74	205.36	52.34	2.89	16.31
	1	a						
		b						
		c						
	2	a	246.01	33.51	212.50	52.22	6.47	16.63
		b	263.73	27.28	236.45	53.82	5.43	20.04
		c	278.01	31.86	246.15	51.56	6.29	16.52
	3	a	299.52	89.42	210.10	49.91	16.51	16.54
		b	340.75	102.37	238.37	50.56	19.46	16.81
		c	335.17	96.73	238.45	52.70	17.98	17.97
	4	a	514.46	183.17	331.29	47.24	22.45	24.28
		b	496.81	164.03	332.78	48.10	21.68	26.01
		c	519.85	184.59	335.26	51.67	21.84	
	5	a	593.98	308.74	285.24	48.68	27.86	20.44
		b	583.30	311.79	271.51	48.91	29.19	19.12
		c	610.89	304.18	306.72	49.97	26.18	
	6	a	631.70	295.30	336.40	49.08	28.21	20.52
		b	628.79	301.80	326.99	52.58	27.40	24.83
		c	634.56	256.02	378.54	49.62	23.80	24.24
	7	a	629.65	297.85	331.80	48.73	26.76	21.63
		b	607.25	302.92	304.33	53.17	27.73	25.13
		c	608.15	273.98	334.17	51.33	26.71	24.24
	8	a						
		b						
		c						
2	0	a	71.30	22.62	48.68	41.85	3.59	4.39
		b	71.30	21.78	49.51	42.80	3.45	4.97
		c	70.47	22.57	47.90	43.33	3.77	5.20
	1	a						
		b						
		c						
	2	a	96.87	45.74	51.13	44.28	7.19	6.81
		b	96.26	43.03	53.23	41.79	6.87	4.20
		c	97.28	46.12	51.17	42.45	6.71	5.27
	3	a	126.12	74.68	51.44	43.81	13.69	7.63
		b	133.45	72.32	61.13	43.93	13.28	
		c	150.97	75.61	75.37	43.04	13.69	7.36
	4	a	282.47	210.03	72.44	40.91	28.60	11.53
		b	263.03	210.04	52.99	40.39	29.14	10.43
		c	306.28	206.54	99.73	46.55	30.07	
	5	a	536.24	346.41	189.83	39.47	28.99	9.93
		b	544.07	393.58	150.49	39.93	30.58	8.83
		c	525.94	313.31	212.63	42.12	28.16	
	6	a	566.38	369.33	197.05	40.31	28.10	11.80
		b	581.57	347.02	234.55	39.66	28.21	11.08
		c	562.89	308.95	253.93	40.55	26.66	13.52
	7	a	616.90	381.77	235.13	40.96	27.41	13.19
		b	599.94	307.02	292.93	40.67	26.43	13.94
		c	573.91	325.04	248.86	39.30	27.24	11.69
	8	a	622.46			44.40	24.70	19.40
		b	613.93	275.10	338.83	40.79	23.32	16.69
		c	557.37	287.95	269.42	45.70	25.40	20.03

Incubation Number	Day	Replicate	TOC (μM)	POC (μM)	DOC (μM)	TN (μM)	PON (μM)	DON (μM)
3	0	a	57.94	7.13	50.81	41.62	1.05	6.70
		b	56.70	6.71	49.98	40.85	1.12	5.01
		c	58.65	6.92	50.40	41.79	1.09	5.86
	1	a						
		b						
		c						
	2	a	61.91	9.89	52.02	40.73	1.46	5.50
		b	62.73			41.62	1.80	5.86
		c	54.94	10.66	44.28	40.79	1.49	5.97
	3	a	67.31	20.02	47.30	40.96	2.98	5.90
		b	66.65	21.19	45.46	40.79	3.38	4.60
		c	65.42	16.31	49.11	45.70	2.87	9.64
	4	a	75.03	33.68	41.35	40.13	5.90	6.15
		b		31.16		40.43	6.04	5.37
		c	79.34	29.18	50.16	40.49	6.13	5.40
	5	a	187.47			37.59		
		b	173.54	109.42	64.11	39.66	21.05	8.11
		c	185.78	128.40	57.38	38.83	24.91	6.47
	6	a	459.48	209.48	249.99	38.59	21.70	16.39
		b	363.47	190.25	173.22	38.59	23.56	14.05
		c	402.81				27.64	
	7	a	459.33	256.83	202.50	33.72	26.24	7.03
		b	370.94	253.33	117.61	34.72	28.98	4.73
		c	452.80	237.08	215.72	35.08	26.47	7.96
	8	a	482.20	288.70	193.50	33.51	26.30	6.66
		b	378.62			34.87		
		c	468.76	265.42	203.34	35.55	27.25	8.07
4	0	a	57.03	5.45	51.58	32.73	0.89	6.85
		b	56.78	4.61	52.18	33.85	0.78	7.68
		c	64.18	4.33	59.85	33.08	0.80	
	1	a						
		b						
		c						
	2	a	64.13			32.85		
		b	62.55	5.42	57.13	34.09	1.05	8.05
		c	61.40	5.47	55.93	34.39	0.89	9.05
	3	a	61.02	8.29	52.72	33.44	1.60	8.38
		b	60.99	7.83	53.16	33.85	1.60	7.80
		c	58.25	8.12	50.13	32.73	1.57	7.35
	4	a	63.91	19.01	44.90	28.96	3.63	3.35
		b	65.92	21.92	44.00	30.32	3.97	
		c	68.98	19.87	49.11	29.80	3.76	4.36
	5	a	90.43	54.50	35.93	29.74	10.52	5.95
		b	101.51	67.43	34.08	30.32	12.45	5.63
		c	95.91	66.56	29.36	29.33	12.25	5.87
	6	a	242.79	183.90	58.89	28.54	19.64	8.65
		b	280.49	217.04	63.45	29.12	20.28	8.57
		c	258.34	201.96	56.38	27.76	19.17	
	7	a	329.59	229.28	100.31	28.80	20.05	8.50
		b	347.22	266.67	80.56	28.86	22.10	6.51
		c	344.24	238.98	105.26	27.81	20.69	6.80
	8	a						
		b						
		c						

Incubation Number	Day	Replicate	NO_3^- (μM)	NO_2^- (μM)	NH_4^+ (μM)	$Si(OH)_4$ (μM)	PO_4 (μM)
1	0	a	28.44	0.27	1.20	39.33	2.48
		b	31.78	0.30	1.36	45.72	2.69
		c	32.15	0.30	0.69	45.98	2.75
	1	a					
		b					
		c					
	2	a	28.72	0.36	0.05	41.99	2.44
		b	27.98	0.35	0.02	41.19	2.40
		c	28.36	0.35	0.04	40.66	2.40
	3	a	16.37	0.41	0.07	32.67	1.65
		b	13.76	0.40	0.14	30.01	1.45
		c	16.11	0.40	0.24	32.14	1.60
	4	a	0.00	0.12	0.39	1.65	0.48
		b	0.00	0.13	0.28	5.23	0.46
		c	0.00	0.12		3.32	0.45
	5	a	0.00	0.13	0.25	0.82	0.78
		b	0.23	0.13	0.24	11.01	0.67
		c				0.05	0.64
	6	a	0.00	0.07	0.29	0.20	0.74
		b	0.00	0.07	0.28	2.34	0.73
		c	0.00	0.07	1.52	0.72	0.64
	7	a	0.00	0.07	0.26	0.18	0.69
		b	0.00	0.05	0.26	0.00	0.72
		c	0.00	0.06	0.33	0.00	0.70
	8	a					
		b					
		c					
2	0	a	30.67	0.37	2.82	48.38	3.00
		b	31.14	0.38	2.87	48.65	3.02
		c	31.14	0.38	2.85	48.91	3.02
	1	a					
		b					
		c					
	2	a	28.87	0.40	1.01	43.59	2.73
		b	29.23	0.41	1.08	44.65	2.79
		c	28.67	0.41	1.38	43.32	2.78
	3	a	22.00	0.42	0.07	35.07	2.17
		b		0.41	0.05	35.33	2.07
		c	21.43	0.42	0.14	35.33	2.09
	4	a	0.21	0.14	0.42	10.47	0.64
		b	0.31	0.14	0.37	11.85	0.64
		c				9.43	0.67
	5	a	0.00	0.15	0.41	1.14	0.78
		b	0.00	0.15	0.36	1.42	0.69
		c	0.00			1.21	0.80
	6	a	0.00	0.07	0.34	0.44	0.73
		b	0.00	0.07	0.30	0.44	0.57
		c	0.00	0.08	0.29	1.23	0.76
	7	a	0.00	0.06	0.30	0.43	0.64
		b	0.00	0.06	0.24	1.62	0.67
		c	0.00	0.05	0.33	0.96	0.69
	8	a	0.00	0.06	0.24	0.41	0.55
		b	0.00	0.05	0.72	1.48	0.77
		c	0.00	0.04	0.23	2.16	0.83

Incubation Number	Day	Replicate	NO_3^- (μM)	NO_2^- (μM)	NH_4^+ (μM)	$Si(OH)_4$ (μM)	PO_4 (μM)
3	0	a	33.72	0.14	0.00	49.18	2.77
		b	34.52	0.19	0.00	49.18	2.81
		c	34.56	0.15	0.00	49.71	2.83
	1	a					
		b					
		c					
	2	a	33.62	0.15	0.00	49.18	2.76
		b	33.77	0.19	0.00	48.91	2.76
		c	32.66	0.17	0.49	44.65	2.73
	3	a	31.86	0.19	0.04	49.35	2.52
		b	32.47	0.22	0.12	49.08	2.56
		c	32.93	0.21	0.06	50.19	2.61
	4	a	27.92	0.15	0.01	47.01	2.21
		b	28.73	0.19	0.11	46.74	2.29
		c	28.74	0.17	0.05	47.80	2.33
	5	a	3.26	0.14	0.13	29.61	0.72
		b	10.07	0.26	0.17	36.88	1.13
		c	7.04	0.23	0.18	37.15	1.00
	6	a	0.00	0.07	0.44	12.12	0.64
		b	0.00	0.06	0.92	21.54	0.75
		c	0.00	0.06	0.38	17.50	0.68
	7	a	0.00	0.05	0.40	8.08	1.21
		b	0.00	0.05	0.95	14.00	0.92
		c	0.00	0.05	0.59	10.77	1.04
	8	a	0.00	0.05	0.50	6.47	0.97
		b	0.00	0.05	0.15	11.85	0.86
		c	0.00	0.05	0.18	9.43	0.85
4	0	a	24.75	0.15	0.09	29.30	2.00
		b	25.20	0.15	0.03	29.59	2.01
		c		0.14		29.05	1.96
	1	a					
		b					
		c					
	2	a		0.16		27.69	1.87
		b	24.82	0.17	0.00	29.63	1.97
		c	24.29	0.16	0.00	28.81	1.95
	3	a	23.27	0.18	0.00	28.00	1.89
		b	24.26	0.18	0.00	29.38	1.94
		c	23.63	0.18	0.00	28.84	1.91
	4	a	21.74	0.12	0.12	28.26	1.77
		b			0.00	27.46	1.70
		c	21.56	0.12	0.00	28.53	1.76
	5	a	13.08	0.13	0.06	24.77	1.20
		b	12.06	0.16	0.02	26.38	1.17
		c	11.00	0.14	0.06	23.42	1.12
	6	a	0.00	0.06	0.20	13.52	0.41
		b	0.00	0.06	0.20	13.24	0.37
		c	0.00	0.05		13.24	0.37
	7	a	0.00	0.06	0.19	8.15	0.46
		b	0.00	0.06	0.19	8.89	0.56
		c	0.00	0.06	0.25	8.14	0.50
	8	a					
		b					
		c					

Incubation Number	Day	Replicate	> 20 μm Chl a (μg L^{-1})	3 to 20 μm Chl a (μg L^{-1})	< 3 μm Chl a (μg L^{-1})
1	0	a	2.85	0.53	0.16
		b	2.98	0.56	0.16
		c	2.92	0.56	0.16
	1	a			
		b			
		c			
	2	a	6.10	0.41	0.11
		b	6.26	0.49	0.13
		c	5.85	0.49	0.09
	3	a	15.82	1.22	0.24
		b	19.15	1.18	0.25
		c	15.39	1.43	0.20
	4	a	21.03	1.66	0.29
		b	22.10	1.99	0.34
		c	19.73	2.31	0.31
	5	a	18.36	1.21	0.32
		b	19.78	1.42	0.31
		c	20.83	1.28	0.31
	6	a	11.41	1.22	0.62
		b	12.79	1.23	0.47
		c	12.28	1.02	0.60
	7	a	8.62	0.69	0.62
		b	9.57	0.77	0.64
		c	8.89	0.96	0.77
	8	a			
		b			
		c			
2	0	a	1.85	0.94	0.39
		b	1.87	0.82	0.38
		c	2.05	1.09	0.41
	1	a			
		b			
		c			
	2	a	5.68	0.88	0.11
		b	7.01	0.89	0.11
		c	5.54	1.09	0.11
	3	a	15.97	2.87	0.30
		b	14.81	2.85	0.27
		c	13.61	3.13	0.30
	4	a			0.83
		b	30.87	7.70	0.74
		c	32.15	8.09	0.77
	5	a	24.00	6.02	0.78
		b	23.99	5.86	0.75
		c	23.54	5.70	0.79
	6	a	19.40	2.90	0.71
		b	19.92	2.55	0.73
		c	15.73	2.16	0.73
	7	a	19.75	1.68	0.52
		b	12.89	1.13	0.51
		c	13.37	1.33	0.60
	8	a	17.42	0.93	0.52
		b	12.65	0.95	0.40
		c	13.81	0.82	0.58

Incubation Number	Day	Replicate	> 20 μm Chl a (μg L^{-1})	3 to 20 μm Chl a (μg L^{-1})	< 3 μm Chl a (μg L^{-1})
3	0	a	0.04	0.26	0.10
		b	0.16	0.15	0.11
		c	0.17	0.14	0.09
	1	a			
		b			
		c			
	2	a	0.62	0.09	0.08
		b	0.28	0.18	0.10
		c	0.34	0.14	0.07
	3	a	1.16	0.23	0.19
		b	1.19	0.42	0.24
		c	1.13	0.29	0.18
	4	a	4.67	0.72	0.40
		b	4.22	1.19	0.48
		c	4.55	0.98	0.39
	5	a	21.74	2.40	1.33
		b	11.49	3.58	1.05
		c	18.10	2.36	0.95
	6	a	13.05	3.53	0.97
		b	10.78	4.58	1.21
		c	15.14	3.91	1.03
	7	a	6.84	1.62	0.97
		b	6.42	2.75	1.10
		c	7.42	2.65	1.17
	8	a	5.57	1.43	1.18
		b	4.83	3.20	1.20
		c	5.02	1.84	1.23
4	0	a	-0.06	0.14	0.05
		b	-0.05	0.11	0.05
		c	-0.01	0.09	0.05
	1	a			
		b			
		c			
	2	a	-0.52	0.69	0.08
		b	-0.16	0.52	0.07
		c	-0.16	0.36	0.07
	3	a	-0.07	0.59	0.17
		b	0.19	0.55	0.17
		c	0.20	0.62	0.18
	4	a	0.79	1.98	0.52
		b	0.85	2.10	0.55
		c	1.00	2.01	0.55
	5	a	2.47	6.92	1.22
		b	3.58	7.73	1.51
		c	3.83	6.46	1.46
	6	a	6.67	16.68	1.81
		b	6.61	16.75	1.99
		c	9.98	14.15	1.80
	7	a	4.81	11.52	1.14
		b	3.70	13.61	1.08
		c	1.04	12.62	1.12
	8	a			
		b			
		c			

Incubation Number	Day	Replicate	HNA Bacteria Abundance (x 10^6 cells ml^{-1})	LNA Bacteria Abundance (x 10^6 cells ml^{-1})	Synechococcus Abundance (x 10^3 cells ml^{-1})	Synechococcus Fluorescence $cell^{-1}$
1	0	a	0.50	0.20	1.60	578.19
		b	0.53	0.20	1.55	528.12
		c	0.55	0.17	1.27	575.83
	1	a				
		b				
		c				
	2	a	0.74	0.23	1.82	377.63
		b	0.89	0.30	2.02	412.89
		c	0.75	0.30	1.98	404.41
	3	a	1.34	0.41	3.08	389.47
		b	1.37	0.32	2.87	409.47
		c	1.57	0.33		436.71
	4	a	3.06	0.42	4.04	327.61
		b	3.08	0.41	2.91	383.12
		c	3.10	0.42		296.45
	5	a	11.14	0.95	6.23	210.65
		b	13.84	0.87	4.87	203.58
		c	11.96		6.29	192.81
	6	a	5.15	0.56	6.46	124.4
		b				119.1
		c	5.17	0.50	5.32	129.3
	7	a	2.23		3.45	171.16
		b	1.86	0.25	2.97	154.84
		c	2.13	0.20	3.35	
	8	a				
		b				
		c				
2	0	a	0.89	0.24	2.86	669.81
		b	0.69	0.22	2.47	703.13
		c	0.79	0.27	2.28	740.77
	1	a				
		b				
		c				
	2	a	1.39	0.35	6.45	391.91
		b	1.24	0.33	6.15	376.15
		c	1.25	0.28	5.97	471.33
	3	a	1.49	0.32	13.65	343.08
		b	1.51	0.30	13.50	327.44
		c	1.49	0.30	12.80	299.51
	4	a	1.51	0.33	23.41	226.06
		b	1.90	0.37	20.63	212.03
		c	1.88	0.33	21.50	193.27
	5	a	2.96		31.93	108.38
		b	3.88	0.30	33.42	97.62
		c		0.32	26.02	87.65
	6	a	4.03	0.22	32.04	55.46
		b	3.30	0.27		53.32
		c			37.79	45.3
	7	a	2.28	0.12	19.99	61.21
		b		0.12	22.66	57.2
		c	2.55	0.16	22.27	52.77
	8	a	2.12	0.16	17.50	69.11
		b	2.71	0.15	18.56	73.56
		c	2.33	0.12	19.99	71.67

Incubation Number	Day	Replicate	HNA Bacteria Abundance (x 10^6 cells ml^{-1})	LNA Bacteria Abundance (x 10^6 cells ml^{-1})	Synechococcus Abundance (x 10^3 cells ml^{-1})	Synechococcus Fluorescence $cell^{-1}$
3	0	a	0.48	0.19	2.56	494.81
		b	0.40	0.15	2.44	424.56
		c				380.97
	1	a				
		b				
		c				
	2	a	0.81	0.21	3.92	253.71
		b	0.93	0.24		295.98
		c	0.80	0.22	3.30	253.93
	3	a	0.96	0.22	5.52	221.05
		b	1.11	0.25		247.6
		c	0.96	0.26	5.13	235.97
	4	a	1.06	0.23	10.54	217.55
		b	1.22	0.25	12.07	216.29
		c	1.05	0.28		198.09
	5	a	1.25		15.46	186.53
		b	1.00	0.26		205.94
		c	1.33	0.31	15.77	193.17
	6	a		0.30	20.19	
		b	3.88	0.37		139.6
		c	4.06	0.39	19.27	113.39
	7	a	9.57	0.29	26.32	55.56
		b	11.02	0.33		54.18
		c		0.30	28.56	44.08
	8	a		0.20	12.10	34.98
		b	1.92			
		c	2.49	0.15	7.71	48.25
4	0	a	0.37	0.22	6.07	192.93
		b	0.40	0.24	6.83	211.04
		c	0.40	0.23	5.87	189.23
	1	a				
		b				
		c				
	2	a	0.52	0.23	7.58	368.96
		b	0.50	0.24	8.23	346.68
		c	0.52	0.24	9.24	385.23
	3	a	0.65	0.30	11.82	300.33
		b	0.78	0.27	11.90	361.94
		c	0.66	0.26	12.90	345.13
	4	a	0.89	0.25	15.52	373.89
		b	1.06	0.24	16.06	395.38
		c	0.81	0.22	17.12	448.54
	5	a	1.04		26.33	352.55
		b	1.24	0.33	34.71	364.91
		c	1.07	0.36	30.47	317.73
	6	a	1.66	0.30	63.75	153.07
		b	1.60	0.32	62.99	122.82
		c	1.55	0.31	60.93	142.4
	7	a	4.24	0.33		41.36
		b	4.31	0.37	59.24	38.72
		c	4.77	0.40	64.65	41.42
	8	a				
		b				
		c				

Incubation Number	Day	Replicate	Sm Euk Phyto Abundance (x 10^3 cells ml^{-1})	Sm Euk Phyto Fluorescence $cell^{-1}$	Lg Euk Phyto Abundance (x 10^3 cells ml^{-1})	Lg Euk Phyto Fluorescence $cell^{-1}$
1	0	a	0.24	1315.74		
		b		1049.11		
		c	0.20	1226.94		
	1	a				
		b				
		c				
	2	a	0.60	1049.75		
		b		1326.44		
		c	0.71	1160.74		
	3	a	2.75	1123.46		
		b	3.12	1079.59		
		c		1166.77		
	4	a	2.94			
		b	5.02	1073.73		
		c		968.58		
	5	a				
		b	4.40	1166.08		
		c	5.66	1154.7		
	6	a	16.31	633.26		
		b	16.06	630.01		
		c	18.52	680.07		
	7	a	7.26	1257.63		
		b	8.11	1072.47		
		c		1275.14		
	8	a				
		b				
		c				
2	0	a	1.06	862.27	0.85	9089.69
		b	1.03	978.58	0.84	9104.76
		c	0.90			9087.28
	1	a				
		b				
		c				
	2	a	2.04	1913.34	3.68	8319.70
		b	1.71	1921.53	3.33	8318.67
		c	2.07	1963.91	3.76	8342.00
	3	a	8.51	1653.49	15.36	8576.84
		b		1950.55	12.82	8532.93
		c	8.88	1694.28	13.82	8588.88
	4	a	39.61	804.4	69.52	8044.90
		b	43.00	785.13		7941.76
		c	40.82	801.23	62.84	7822.60
	5	a	63.09	331.2	85.51	6540.38
		b	61.40	360.16	80.75	6846.00
		c		350.55	64.50	6348.81
	6	a	27.76	332.04	53.95	6362.44
		b	32.44	304.84	55.24	5242.64
		c	34.09	321.28	45.10	5453.66
	7	a	9.46	290.7	37.37	5717.91
		b	10.03	308.18	31.59	5409.91
		c	8.87	315.54	39.82	5133.72
	8	a	4.62	297.11	26.53	5776.46
		b	4.87	295.04	24.13	5506.82
		c	4.16	329.19	26.27	5357.66

Incubation Number	Day	Replicate	Sm Euk Phyto Abundance (x 10^3 cells ml^{-1})	Sm Euk Phyto Fluorescence $cell^{-1}$	Lg Euk Phyto Abundance (x 10^3 cells ml^{-1})	Lg Euk Phyto Fluorescence $cell^{-1}$
3	0	a	0.56		0.70	8650.73
		b	0.49	1071.98	0.47	9209.08
		c		847.61		9451.72
	1	a				
		b				
		c				
	2	a	1.75	928.03	0.43	8798.79
		b	1.75	909.41	0.43	9006.11
		c		850.4		8402.10
	3	a	3.46	817.19	1.08	8513.04
		b	3.19	988.16		8710.71
		c	2.76	867.93	1.13	8902.98
	4	a	16.16	534.29	5.55	8120.95
		b		515.19	4.58	8233.74
		c	14.87	551.3	5.03	8458.04
	5	a	51.54	473.09	27.35	8498.73
		b	55.77	407.36	24.34	7968.80
		c	59.21	416.51	24.89	8149.99
	6	a		275.26	91.87	4354.49
		b	71.49	308.86	75.62	4805.50
		c	77.67	313.95	69.25	5004.06
	7	a	88.25	284.57	59.31	4867.29
		b	90.05	283.29		4783.38
		c	77.07	271.7	62.60	4361.78
	8	a	64.35	274.14	61.69	4691.99
		b	52.46	211.63		4021.29
		c		229.81	55.83	4369.53
4	0	a		1227.96		6667.51
		b	0.68	1226.05	0.12	6922.83
		c	0.70	1268.62	0.08	7100.30
	1	a				
		b				
		c				
	2	a	1.25	1034.94		8702.25
		b	1.22		0.32	8267.87
		c	0.95	1220.33	0.36	8992.33
	3	a	4.82	1096.87	0.72	8879.16
		b	4.34	1141.61	0.92	8736.14
		c	5.12	1006.2	0.91	8929.33
	4	a	8.64	1337.41	2.09	8842.77
		b	7.38	1337.63		8583.82
		c	7.04	1452.02	1.83	8591.94
	5	a	26.90	1144.46		8928.27
		b		1126.9	9.48	8948.38
		c	34.31	1124.35	8.50	8791.36
	6	a	145.32	643.78	21.35	8510.07
		b	143.64	735.08	22.03	8621.91
		c	144.83	745.46	22.77	8720.30
	7	a		609.97		7933.34
		b	122.26	769.38	45.11	8031.12
		c	131.03	710.81	47.54	7351.60
	8	a				
		b				
		c				